PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter

November 4, 2015

Research Emphasis Day

AUBURN UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

PHI ZETA
EPSILON CHAPTER
COLLEGE OF VETERINARY MEDICINE
AUBURN UNIVERSITY

welcomes you to our

PHI ZETA RESEARCH DAY
November 4, 2015

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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PROGRAM

PHI ZETA RESEARCH DAY FORUM

NOVEMBER 4, 2015 – VETERINARY EDUCATION CENTER

8:30: BREAKFAST Buffet - VEC Lobby

9-11:12 MORNING Presentations - Overton Auditorium

Undergraduate Students

9:00	Lauren Ellis	Increased lifespan in a new mucopolysaccharidosis-like variant of feline GM1 gangliosidosis

Veterinary Students

9:12	Amanda Crouthamel	Comparison of Acetaminophen Administration to Horses through Syringe and Nasogastric Tube
9:24	Patrick Jones	Effect of Abdominal Insufflation on Direct Intra-Abdominal Pressure and Medial Saphenous Venous Pressure in Sedated Horses
9:36	Annie Maguire	Myelin Alterations in a Feline Neurodegenerative Disease
9:48	Emily Nielsen	Evaluation of the Follicular Cycle in Ball Pythons (Python regius)

Graduate Students

10:00	Marta Barba	*Corynebacterium pseudotuberculosis* seroprevalence in healthy horses in a non-endemic state
10:12	Jenna Bayne	Use of accelerometers to monitor behavior changes in beef cattle during the periparturient period
10:24	Noelle Bergman	Efficacy of vinblastine as front line therapy for canine patient with multicentric lymphoma
10:36	Erfan Chowdhury	A synthetic biodegradable microsphere vaccine of femtomole-dosed peptide antigens protects better against *Chlamydia abortus* than previous infection
10:48	Matthew Coleridge	Effect of Biological Media on Knot Security
11:00	Fatma Eldemery	Characterization of Binding of Infectious Bronchitis Virus Spike Proteins Representing Vaccine Subpopulations to Chicken Tissues

11:12–12:30 POSTER Presentations- VEC Lobby

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

1:00-3:15 AFTERNOON Presentations - Overton Auditorium

Graduate Students (continued)

1:00	Saiada Farjana	Changes in avian infectious bronchitis virus (IBV) spike proteins associated with adaptation to chicken embryonic kidney (CEK) cells do not improve attachment
1:12	Alex Gillen	*In Vitro* Evaluation of the Forwarder Knot
1:24	Heather Gossett	Pilot Study: Evidence of a Hemp Based Product as Therapeutic Intervention for Treatment of Navicular Disease in Horses
1:36	Laura Haysom	Evaluation of Histogel™ and Gelfoam™ embedded bronchoalveolar lavage and transtracheal wash specimens in comparison to cytospin and sediment smear preparations
1:48	Rochelle Jensen	Adenoviral Vectored Gonadotropin Releasing Hormone Vaccine for Estrus Suppression in the Mare
2:00	Steven Kitchens	Factors influencing environmental Salmonella incidence and proximal movement in a multi-species animal facility
2:12	Thibaud Kuca	Genomic Change of Bovine Viral Diarrhea Virus (BVDV) by Serial Infections in Sheep
2:24	J. Forrest Shirley	Characterization and Simplification of a Bacteriophage Cocktail to Reduce Salmonella Lymph Node Carriage in Calves
2:36	Wei Wang	Characterization of nine novel naturally occurring melanocortin-4 receptor mutations
2:48	Randolph Winter	Treatment with Endothelial Colony Forming Cells (ECFCs) and PEG-fibrinogen Encapsulated ECFCs Reduces Wound Surface Area in Equine Distal Limb Wounds

Post-graduate/Faculty

3:00	Amanda Taylor	Successful Use of Stereotactic Image-Guided Biopsies in 5 dogs

3:15-4:00: View Posters, Snack - VEC Lobby

4:00: KEYNOTE LECTURE

Additive Manufacturing: Great Potential, New Challenges and Opportunities

Dr. Warren Haggard
Associate Dean for Engineering Research, University of Memphis

Dr. Warren O. Haggard is the Associate Dean of Research and Graduates for the Herff College of Engineering and a Professor and Herff Chair of Excellence in Biomedical Engineering at the University of Memphis. Dr. Haggard has a BS degree in Chemistry from Auburn University and MSE and Ph.D. in Biomedical Engineering from University of Alabama at Birmingham. His research interests are focused on bone graft substitutes, functional tissue repair and local drug delivery for complex extremity trauma with emphasis on musculoskeletal applications and he has 80 publications, 1 published book, over 120 presentations and 7 patents. Dr. Haggard's current investigations are local antibiotic delivery to prevent bone infections, intelligent drug delivery systems, and functional musculoskeletal hard/soft tissue repair. Dr. Haggard has extensive past applied research and development experiences as a medical device industrial engineer and vice president in industrial research for over 18 years and he developed or helped develop over 25 clinically successful products. He has translated two technologies from his university laboratory to local medical device companies.

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

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

5:30 Doors open

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

7:00 INDUCTION of new Phi Zeta Members

AWARD CEREMONY to honor winners of the Platform and Poster Competitions

Posters

Undergraduate Students

Jessica Isaac	Evaluation of In Vitro Cross-neutralization between Bovine Parainfluenza 3 Virus Genotypes
Isabelle Kallenberg	Pathogen Associated Molecular Pattern (PAMP) Receptor Expression in Chickens Before and After IBV Challenge

Veterinary Students

Kelsi Anderson	Circulating Plasma miRNAs as Novel Biomarkers in Congestive Heart Failure
Jordan Ayers	Validation and Genetic Profiling of Canine Mammary Tumor Receptor Gene Expression
Elizabeth Gammon	Monitoring Humoral Immune Response to Viral Vector and Osteosarcoma Tumor Antigen
Megan Hataway	Redox-Responsive MRI Contrast Agents for the Detection of Doxorubicin-Induced Oxidative Stress in the Heart
Lyndsey Hayden	Friction Measurements of Intact Equine Carpal Articular Cartilage
Rebecca Kennerly	Identification and characterization of Glanzmann Thrombasthenia in a beagle-mix dog
Samantha Morici	Search for a Pan-Tumor Promoter for Use in Conditionally Replicative Adenoviral Vectors
Nichole Murdock	Evaluation Of An Aqueous Extract Of Terminalia Chebula For Anti-Arthritic Efficacy And Safety In Osteoarthritic Dogs: Radiographic Evidence
Jaida Reeves	The Impact of Analgesic and Anesthetic Drugs on Sperm Motility in Equine and Bovine Species
Charles Rehm	The Pharmacokinetics of Altrenogest (Regu-Mate®) in Lactating Mares and Suckling Foals
Camille Roemhild	Thru-Hiking the Appalachian Trail: The Effect of Motivation and Personality on Successfully Accomplishing Long-Term Goal
Kathleen Stewart	Mitophagy and Histone Deacetylase Inhibitory Activity of Small Molecule Carnitinoid Antioxidant Compounds
Emily Velez	*In Vitro* Sensitivity of Canine Glioblastoma Cells to Temozolomide

Niloofar Yeganeh	Age-Dependent Memory Response against Live Attenuated Infectious Bronchitis Virus Vaccine in Chickens

Graduate Students

Jessica Bailey	Concurrent Cutaneous Phaeohyphomycosis And Nocardiosis In A Dog
Eric Fish	Characterization and microRNA profiling of canine mammary tumor exosomes
Michelle Hoffman	Creating a Reproducible and Quantifiable Behavioral Model of Mitochondrial Dysfunction in the Rat
Serene Lai	*In vitro* anti-tubulin effects of benzimidazole anthelmintics mebendazole and fenbendazole on canine glioblastoma cells
Gisela Martinez-Romero	Expression of Mammaglobin-A in canine tumors
Mariano Mora Pereira	Sustained-Release Voriconazole Hydrogel for Ocular Use in Horses: Safety and *in vivo* studies
Roxy Rodriguez Galarza	Evaluation of commercial glucometer test strip for measurement of glucose in tears and potential correlation with blood glucose in dogs
Abdul Mohin Sajib	Genetic Modification to Achieve Targeting of Adenoviral Vectors to Malignant Cells of Lymphocyte Origin
Randolph Winter	Growth and Function of Equine Endothelial Progenitor Cells Labeled with Semiconductor Quantum Dots

Post-graduate/Faculty

Payal Agarwal	Evaluation of a conditionally replicative adenoviral vector for the treatment of canine osteosarcoma
J. W. Koehler	A Novel Inherited Cerebellar Abiotrophy in a Cohort of Related Goats
Xiaoqiang Liu	Multilocus sequence typing and virulence profiles in uropathogenic *Escherichia coli* isolated from cats in the United States
Benjamin Newcomer	Evaluation of Delayed Insemination with Sexed Semen for Non-responding Beef Heifers after Estrus Synchronization

Undergraduate Student Platform Presentations

Increased lifespan in a new mucopolysaccharidosis-like variant of feline GM1 gangliosidosis.

Lauren Ellis[1], Heather Gray-Edwards[1], Amanda Gross[1], Ashley Randle[1], Adrien Hespel[2], Seung-Woo Jung[3], Brandon Brunson[3], Patricia Beadlescomb[1], Nouha Salibi[4], Ronald Beyers[5], Thomas Denney[5] and Douglas R. Martin[1,3]

[1]Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL; [2]Department of Clinical Sciences, College of Veterinary Medicine, University of Madison, WI; [3]Department of Clinical Sciences Auburn CVM. [4]Department of Anatomy, Physiology and Pharmacology, Auburn University, AL; [5]Siemens Healthcare, Malvern, PA, United States; [6]AU MRI Research Center, College of Engineering, Auburn University, AL

Introduction. GM1 gangliosidosis is a fatal neurodegenerative disorder of children. Treatment for this disease is restricted to palliative care and patients with the infantile form survive until ~2 years of age. GM1 is caused by a mutation in the gene encoding β-galactosidase (GLB-1), resulting in storage of GM1 ganglioside, oligosaccharides and glycosaminoglycans (GAGs). Feline GM1 gangliosidosis was discovered ~30 years ago and recapitulates human disease. The feline GM1 colony has been meticulously maintained since its discovery and has proved invaluable in the development of novel therapeutics. Recently, a new phenotype has been discovered in the GM1 cat colony. Three GM1 affected kittens presented with a mucopolysaccharidosis (MPS)-like phenotype, with a shortened stature, blunted facial features and an increased lifespan compared to other GM1 affected kittens. The MPS-like GM1 kittens have a mean survival of 10.0±0.2 months compared to GM1 affected kittens at 7.3±0.5 ($p<0.0001$). The new phenotype is remarkably similar to Morquio syndrome in people (MPS IV type B) which is also caused by a different mutation in GLB-1 gene. Therefore, we hypothesize that the MPS-like GM1 kittens have a second mutation in GLB-1 resulting in the new phenotype.

Methods. MRI was performed using a 7 Tesla MAGNETOM scanner (Siemens Healthcare, Erlangen, Germany). Whole body CT was performed using Lightspeed VCT 64 slice CT (GE Healthcare Waukesha, WI). Spinal and limb radiography was performed using the Ysio digital X-ray system (Siemens Medical Solutions Malvern, PA). Echocardiography was performed using a Vivid E9 cardiac ultrasound. Genomic DNA and total RNA were isolated from the liver using the DNeasy and RNeasy (Qiagen, Venlo, Netherlands) kits, respectively and cDNA was made using Super Script III First Strand kit (Invitrogen, Carlsbad, CA). Primers were designed using DNA star (Madison, WI) or obtained from previously published sequences (*Mol. Gen. Met*. 94(2):212-221, 2008). PCR was performed on an iCycler thermocycler (Biorad Hercules, CA).

Results. MRI showed isointensity of the gray and white matter and variable amounts of cortical atrophy consistent with GM1 gangliosidosis, mild spinal cord compression was also noted at C1-2. CT and radiography showed foreshortening of the cervical vertebrae, narrowed and sclerotic physes, bowing of the proximal radius and subluxation of the coxofemoral joints with narrow acetabula. Echocardiography of one cat was within normal limits. Sequencing of GLB-1 revealed a G►C point mutation at 603, a C►T point mutation at 1168, and an A►G point mutation at 1380 that resulted in no amino acid change. Sequencing of the 5' and 3'most ends (100-200 base pairs) of the gene are pending.

Conclusions. MRI, radiographs, and CT support the hypothesis of a concurrent GM1 and MPS-like phenotype. Sequencing thus far does not show a clinically significant mutation in GLB-1 gene of the MPS-like GM1 cats.

Acknowledgments. Scott-Ritchey Research Center, NIH Grants R01HD060576

Graduate Student Platform Presentations

***Corynebacterium pseudotuberculosis* seroprevalence in healthy horses in a non-endemic state.**

Marta Barba[1], Allison J. Stewart[1], Thomas Passler[1], Edzard van Santen[2], and Anne A. Wooldridge[1].

[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]Department of Crop, Soil and Environmental Sciences, College of Agriculture, Auburn University, AL

Introduction. The significance of positive, low antibody titers against *Corynebacterium pseudotuberculosis* in horses remains uncertain. The SHI (synergistic hemolysis inhibition) test has not been rigorously evaluated in a disease-free population.

Methods. Serum SHI test titers from 342 horses from 40 Alabama counties were analyzed. A questionnaire was completed by each owner at the time of blood collection. The association of SHI titers and risk factors was analyzed using a general linear model approach in two steps.

Results. The prevalence of positive antibody titers against *C. pseudotuberculosis* ($\geq$ 1:8) was 52.5% (95% CI, 47-57.9%). Titers $\geq$1:128 were detected in 2.63% (95% CI, 1.2-4.9%) and $\geq$1:512 were detected in 0.3% (95% CI, 0-1.6%) of the sampled population. The factors significantly associated with higher SHI titers were age ($P < 0.001$), breed ($P = 0.023$), and contact with cattle ($P = 0.05$). Contact with goats was associated in the initial but not in the final analysis ($P = 0.19$). Previous travel was not associated with greater SHI titers in the initial model ($P = 0.97$).

Conclusions. The high prevalence of positive titers in a non-endemic population questions the accuracy of the SHI antibody titer test. Possible false positives caused by cross-reaction with antibodies against phospholipases from soil *Corynebacterium* spp. or *C. pseudotuberculosis* biovar *ovis* warrants further investigation. Making clinical decisions only based on SHI testing should be avoided even in non-endemic areas.

Acknowledgments. This research was supported by Boehringer Ingelheim Vetmedica, Inc. We would like to thank the students Bonnie Coats and Brianna Bradford for technical assistance.

Use of accelerometers to monitor behavior changes in beef cattle during the periparturient period

Jenna E. Bayne[1], Paul H. Walz[2], and Thomas Passler[1]

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

Introduction: A large percentage of calf death loss in cow-calf operations occurs during the intrapartum period. The usefulness of commercial activity monitors to detect changes in behavior indicative of impending parturition has not been fully explored. Objectives included characterization of behavioral indices during the periparturient period in beef cattle and the ability of changes in behavioral indices from baseline to accurately predict impending parturition in individual cows.

Methods: Activity data were collected from 40 beef cows housed on pasture using accelerometers (IceQube™, IceRobotics™). Accelerometers were placed on the hind leg of periparturient cows for a minimum of 30 days prior to calving and removed 7 days post-calving. The number of steps, standing time, lying time, and number of lying bouts were continuously recorded at 15 minute intervals throughout the study period.

Results: Evaluation of behavioral data revealed an increase in the steps taken as parturition approached, starting approximately within 2-4 hours prior to calving. Lying bouts became more frequent and shorter in duration as parturition approached as well as demonstrated the most striking deviation from baseline in all the behavioral indices recorded. Immediately following calving, time spent standing increased and lying bout frequency decreased.

Conclusions: Changes in behavioral indices during the periparturient period were demonstrable in beef cattle using accelerometers. The potential exists for the development of algorithms to better predict impending parturition and their application using commercially available activity monitors in pastured beef cattle.

Efficacy of vinblastine as front line therapy for canine patient with multicentric lymphoma

Noelle Bergman[1]; Stephanie Schleis[1]; Annette Smith[1]; William Brawner
[1] Department of Clinical Sciences, Auburn University, AL

Introduction: The efficacy of vinblastine (2mg/m^2) in the treatment of naïve canine lymphoma has not been investigated, yet it is commonly substituted for vincristine in multi-agent chemotherapy protocols. Ideally, drugs suitable for incorporation into multi-agent chemotherapy protocols should have a minimum of 20-30% response rate when used alone. The goal of this study was to determine the response rate of vinblastine in the treatment of naïve canine lymphoma patients.

Methods: Dogs with naïve multicentric lymphoma were recruited. On day 0 complete blood count (CBC), serum chemistry panel, mean sum longest diameter (MSLD) calculations based on peripheral lymph node (LN) measurements, and owner-completed performance status forms were performed. Vinblastine was administered at 2mg/m^2 IV on day 0. On day 7, CBC, MSLD of LNs, and owner completed adverse event forms were performed. VCOG response criteria was used to determine response to vinblastine.

Results: Nine patients were enrolled and 7 patients met inclusion criteria. One patient (14%) achieved a partial response (35.6% decrease in MSLD), 1 patient (14%) experienced progressive disease, and 5 patients (71.4%) experienced stable disease. VCOG grade 1 (n=1), grade 2 (n=1), and grade 3 (n=1) neutropenia was noted. Anorexia (n=2) and lethargy (n=4) were also reported by owners.

Conclusions: Based on this data, vinblastine appears to have minimal efficacy in the treatment of canine multicentric lymphoma and may not be suitable for incorporation into multi-agent protocols. A larger sample size is needed to more definitively assess efficacy.

A synthetic biodegradable microsphere vaccine of femtomole-dosed peptide antigens protects better against *Chlamydia abortus* than previous infection

Erfan Chowdhury[1], Courtney Ober[2], Kh Shamsur Rahman[1], Ram Gupta[2], Bernhard Kaltenboeck[1]

1 Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
2 Department of Chemical Engineering, College of Engineering, Auburn University, AL

Introduction: Successful vaccination against *Chlamydia* spp. has remained elusive, largely due to a lack of vaccine platforms for the required Th1 immunization. Modeling of T helper cell immunity indicates that Th1 immunity requires antigen concentrations that are orders of magnitude lower than those required for Th2 immunity and antibody production. We hypothesized that the *C. abortus* vaccine candidate proteins that we identified earlier, DnaX2, GatA, GatC, Pmp17G, and Pbp3, mediated protection in an A/J mouse model of *C. abortus* lung infection if administered each at 11-90 femtoMoles per mouse. This immunization significantly protected the mice from lethal challenge with 10^8 *C. abortus* organisms. Additional experiments proved that particulate delivery of antigens was required for optimum immunity. We further hypothesized that i) 20-mer peptides overlapping by 10 amino acids could substitute for the whole protein antigens; ii) that release of peptides and adjuvant from 1-10 µm microspheres would enable controlled generation of Th1 immunity; and iii) that inhibition of apoptosis could suppress the inflammatory Th17 response and enhance a protective Th1 response.

Methods: Solutions of peptide antigens with biodegradable poly (lactide-co-glycolide) copolymer (PLGA) and the block copolymer adjuvant Pluronic L121, with or without the apoptosis inhibitor Q-VD-OPH, were spray-dried to 2 µm microspheres, which were administered subcutaneously or intranasally at 10 µg per mouse in a 129S6 mouse model of *C. abortus* lung infection. All mice were challenged intranasally with 3×10^8 *C. abortus* bacteria 6 weeks after vaccination. Naïve, mock-vaccinated mice served as negative controls and mice that received a low intranasal dose of 3×10^7 *C. abortus* bacteria 6 weeks before the high-dose challenge infection served as a control for protective immunity. Ten days post inoculation mice were sacrificed and changes in body & lung weights were determined. *C. abortus* lung loads on day 10 post challenge inoculation were determined by quantitative PCR.

Results: A dose of 2 femtoMoles each peptide per mouse significantly reduced the disease: following lethal challenge the mice did not show any disease symptoms and maintained steady body weight and the lung weights were indistinguishable from the live vaccine protection control. However, this vaccine dose failed to effectively eliminate chlamydiae from the lungs. In contrast, a dose of 0.2 femtoMoles each peptide along with 0.2 µg Q-VD-OPH per mouse generated highly significant protection against *C. abortus* as evidenced by highly significantly reduction in lung's weight and chlamydial burden in comparison to naïve control. Moreover, mice received this vaccine had better body weights than the live vaccine control.

Conclusions: We have developed a fully synthetic biodegradable microsphere vaccine for controlled release of adjuvant and ultralow doses of peptide antigens. This vaccine platform can be used for real-life vaccines as well as a tool to model chlamydial immunopathogenesis by manipulating the vaccine immune response.

Effect of Biological Media on Knot Security

Matthew Coleridge, BEng, BVMS[1]; Amelia Munsterman, DVM, MS, DACVS, DACVECC[1]; Alex Gillen, MA, VetMB, CertAVP, MRCVS[1]; Ramsis Farag PhD[2]; Reid Hanson, DVM, DACVS, DACVECC[1]

1 Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
2 Department of Polymer and Fiber Engineering, Samuel Ginn College of Engineering, Auburn University, AL

Introduction
The Aberdeen knot has been shown to be mechanically superior to surgeon's and square knots with *in vitro*. The objective of this study was to investigate the Aberdeen knot and the effect of 4 biological media (Normosol-R, 1% sodium carboxymethylcellulose (CMC), equine abdominal fat, and equine serum) on its mechanical properties compared to surgeon's and square knots tied with 3 USP polyglactin 910.

Methods
Knots were tied with suture material exposed for 15 minutes to each type of media, or left dry as a control. Pattern ending surgeon's, square and Aberdeen knots were tied in multiple throw combinations. Each combination of knot type, throw number and media exposure was tensed to failure using an INSTRON Universal testing machine. Knot holding capacity (KHC), relative knot security (RKS), knot volume, and weight were determined.

Results
Aberdeen knots were found to have a significantly higher RKS than pattern ending surgeon's ($P<0.0001$) and square ($P<0.0001$) knots irrespective of media exposure. Surgeon's knots had a significantly higher RKS across all media than square knots with a similar number of throws ($P=0.0039$). Irrespective of media exposure, Aberdeen knots were significantly lighter ($P<0.0001$) and smaller ($P<0.0001$) than surgeon's and square knots.

Conculsions
This study concludes that exposure to biological media increases the knot holding capacity of most knots compared to dry knots. Based on *in vitro* knot security and size, Aberdeen knots are the ideal knots to end a continuous pattern.

Characterization of Binding of Infectious Bronchitis Virus Spike Proteins Representing Vaccine Subpopulations to Chicken Tissues

Fatma E. Eldemery, Saiada Farjana, Robert Williams and Vicky L. van Santen

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

Introduction. Infectious bronchitis virus (IBV), an economically important coronavirus of chickens, undergoes an evolutionary process by point mutations, recombination, and selection that results in continuous emergence of new serotypes. Live attenuated ArkDPI-derived vaccines contribute to emergence of new IBV variants in vaccinated chickens by natural selection, resulting in increased vaccine virus virulence and persistence. Previously, five minor vaccine virus subpopulations selected in chickens, designated component (C) (C1-C5), were identified. We hypothesized that spike (S) proteins of these vaccine virus subpopulations that are positively selected in chickens bind more efficiently to chicken tissues than that of the negatively selected major vaccine population. Here we compared spike protein ectodomain (S1+S2) of C2 (strongly selected) and C3 (weakly selected) vaccine subpopulations with major vaccine population (negatively selected) of ArkDPI-derived vaccines in their binding efficiency to different chicken tissues and also determined the effect of the extension of the S1 domain with S2 on binding.

Methods. Secreted, trimeric, strep-tagged recombinant spike proteins representing C2 and C3 vaccine subpopulations as well as the vaccine major population were produced in Human Embryonic Kidney (HEK) 293T cells from codon-optimized (GeneArt) constructs. The proteins were purified using Strep-Tactin® Sepharose columns (IBA GmbH). A tissue array was prepared from healthy 40-day old white leghorn chicken tissues and spike histochemistry was performed for detection of binding of S proteins to chicken tissues. Bound S proteins complexed with Strep-Tactin-HRPO® were visualized with 3-amino-9-ethyl-carbazole (AEC).

Results. C2S1 bound more strongly than vaccine S1 to tracheal tissues and nasolacrimal gland, while C3S1 did not bind to most tissues. None of the tested S1 domains bound to kidney and lung tissues, while all tested ectodomains bound, indicating that S2 is required for binding to these tissues. Addition of S2 allowed the C3 spike protein to bind similarly to the C2 ectodomain, and better than the vaccine ectodomain, to most tissues. By comparing the binding of S1 with ectodomain we found that S2 increases binding efficiency of C2 and vaccine spike proteins and is essential for binding of C3 spike to most tissues.

Conclusions. Spike proteins representing vaccine subpopulations selected in chickens bound better to chicken tissues than that of the negatively selected vaccine major population, suggesting that more efficient viral attachment may contribute to selection of these subpopulations in chickens. The S2 domain may have an important role in attachment and contribute to selection, especially of the weakly selected C3 subpopulation.

Acknowledgments. Financial support was from an Auburn University College of Veterinary Medicine Animal Health and Disease Research grant and the Egyptian Cultural and Educational Bureau. Dr. Toro provided chicken tissues, Dr. Joiner made the tissue array, and Natalia Petrenko, Cindy Hutchinson, Priscilla Barger and Dr. Petrenko lab provided technical assistance. Initial work by Dr. van Santen had technical and financial assistance from members of Dr. Helene Verheije's lab and the Department of Pathobiology, Utrecht University.

Changes in avian infectious bronchitis virus (IBV) spike proteins associated with adaptation to chicken embryonic kidney (CEK) cells do not improve attachment

Saiada Farjana, Fatma Eldemery, Robert Williams and Vicky van Santen
Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Avian Infectious bronchitis virus (IBV), a gamma coronavirus, is one of the most economically important pathogens of chickens and is reportable to the World Organization for Animal Health (OIE). The S1 subunit of the IBV spike (S) protein mediates viral attachment and the S2 subunit is involved in viral fusion to host cells. CEK (chicken embryonic kidney) cells are the only cell culture system for IBV and before propagation the virus must be adapted to CEK cells. Two amino acid changes in the S1 protein and one amino acid change in the S2 protein occurred during adaptation of an IBV Ark serotype vaccine strain to CEK cells (Ghetas, et al., 2015). Besides, our unpublished results showed that adding the S2 domain improved binding of Ark vaccine strain S1 to tracheal tissue in vitro. Based on these observations, we proposed that the alterations in the S gene of CEK-adapted IBV may allow the virus to attach more efficiently to CEK cells compared to Ark-vaccine strains, thus contributing to adaptation. Additionally, binding to CEK cells might also be improved by adding S2 to S1 (whole S protein) rather than using the S1 alone.

Methods. Secreted strep-tagged recombinant S1 or S1+S2 proteins were produced from pCD5 expression vectors transfected into HEK293T (human embryonic kidney) cells as described by Wickramasinghe *et al*., 2011. To obtain constructs expressing CEK-adapted S protein, we introduced the three mutations corresponding to CEK-adapted virus into human codon-optimized vaccine S expression constructs by site directed mutagenesis and overlap extension PCR. Recombinant vaccine and CEK adapted S proteins expressed in HEK293T cells were affinity purified with Strep-Tactin Sepharose columns. For binding assay, spike histochemistry was performed, where purified strep-tagged CEK-adapted and vaccine S1 or S (S1+S2) proteins were complexed with streptactin-HPRO (horse radish peroxidase) and incubated 24 hours with acetone-fixed CEK cells. Then chromogenic substrate AEC was added to detect the bound spike protein. Binding to different formalin-fixed chicken tissues with different S proteins was also evaluated.

Results. We observed no binding to CEK cells and very low binding to fixed choana and nasolacrimal gland by vaccine S1. However, additional/markedly increased binding occurred both to CEK cells and fixed trachea, lung, choana, nasolacrimal gland and cloaca with the whole vaccine S protein, which indicated that whole S protein binds better to both CEK cells and chicken tissues. Surprisingly, rather than the expected improved binding to CEK cells, no binding to CEK cells or fixed chicken tissues occurred with CEK-adapted S protein (either S1 alone or whole S protein).

Conclusions. Our results revealed that inclusion of the S2 subunit improves binding of vaccine S1 to CEK cells. However changes in S protein associated with adaption to CEK cells abolish rather than increase binding to CEK cells. Thus factors other than improved attachment to CEK cells must be involved in adaptation to CEK cells.

Acknowledgments. This investigation was funded by Cellular and Molecular Biology Program and Animal Health and Disease Research Intramural Grant Program, Auburn University. Dr. Toro provided chicken tissues, Dr. Joiner prepared the chicken tissue array, Aly Ghetas prepared primary CEK cells, and Cindy Hutchinson conducted spike histochemistry assays. Krystyna Minc advised on fixation of CEK cells.

In Vitro Evaluation of the Forwarder Knot

Alex Gillen, Amelia Munsterman, Reid Hanson
Department of Clinical Sciences, Auburn University, AL

Corresponding Author: Amelia Munsterman munstas@auburn.edu

Introduction: The forwarder knot is a self-locking knot to start continuous suture patterns. It is used in human bariatric surgery but has not been investigated in suture used in equine surgery.

Methods: Forwarder, surgeon's and square knots were tested using 2USP and 3USP polyglactin 910 and 2USP polydioxanone under linear tension on a universal testing machine recording mode of failure and knot holding capacity (KHC). Relative knot security (RKS) was calculated as a percentage of KHC. Knot volume and weight were assessed by a digital micrometer and balance, respectively. An ANOVA and post hoc testing compared strength between number of throws, suture, suture size, and knot type. $P \leq 0.05$ was considered significant.

Results: Forwarder knots had a higher KHC and RKS than surgeon's or square knots for comparisons of all suture types and number of throws ($P<0.001$). For all suture materials, no Forwarder knots unraveled, but a portion of square and surgeon's knots with under six throws did ($p=0.0407$). Forwarder knots had a smaller volume and weight than both surgeon's and square knots with equal number of throws ($p<0.001$). The knot with a combined highest KHC, RKS, and smallest size and weight was a forwarder of four throws using 3USP polyglactin 910.

Conclusions: The forwarder knots tested were shown *in vitro* to be stronger, more secure and smaller than surgeon's and square knots for starting a continuous suture pattern.

Acknowledgements: Suture was donated by Ethicon, US LLC.:

Pilot Study: Evidence of a Hemp Based Product as Therapeutic Intervention for Treatment of Navicular Disease in Horses

Heather D. Gossett[1], Jennifer S. Taintor[2], Amanda L. Crouthamel, and Dawn M. Boothe[1]

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

Introduction. Recent legalization of marijuana or hemp for medicinal use has been accompanied by increased interest in the implications for veterinary medicine. The most compelling treatment indication relevant to horses is the use of cannabinoids in controlling pain and inflammation. A cannabis supplement comprised of non-psychotropic cannabinoids is being marketed specifically for canine and feline patients for diverse medical indications. Investigating the oral disposition of this product would be beneficial for a wide variety of equine patients including chronic musculoskeletal pain such as seen with navicular disease. The objectives of this study are twofold: to confirm the absence of the psychotropic Δ^9 -tetrahydrocannabinol (Δ^9 –THC) and presence nonpsychotropic cannabinoids (cannabidiol (CBD), cannabichromene (CBC), cannabigerol (CBG), and cannabinol (CBN) in the canine product and the response to treatment after 7 days of dosing of the canine product.

Methods. Three horses were selected for this pilot study. Horses had confirmed diagnosis of navicular syndrome and had not been treated with either oral, injectable, or intra articular medication for the pathology in the last four months. Prior to initiation of the study, the horses were evaluated for baseline lameness using the Lameness Locator. On the day of study initiation, horses were administered their prescribed treatment (13 mg/kg) of cannabinoid supplement "cookies." Horses were dosed twice daily for a total of 7 days. Two hours after dosing the animal, and just prior to the second dosing of the day, 10 mls of whole blood was collected from alternating external jugular veins through venipuncture for evaluation using the investigators validated cannabinoid HPLC assay. Once daily, horses were evaluated using the Lameness Locator to determine whether or not there was an improvement of lameness over the course of treatment.

Results. No adverse events were noted after administration of the cannabinoid supplement to horses. Product administered had detectable levels of nonpsychotropic cannabinoids and was absent of Δ^9 -tetrahydrocannabinol (Δ^9 –THC). CBC was the only detectable cannabinoid in equine serum. There was not a statistically detectable change in lameness in the horses.

Conclusions. These results suggest that the cannabinoids undergo first pass metabolism in the horse, as has been illustrated in other species. These findings support the need for a complete dose titration study and characterization of cannabinoid disposition in horses.

Acknowledgments. Product administered to horses was supplied by Canna-Pet®.

Evaluation of Histogel™ and Gelfoam™ embedded bronchoalveolar lavage and transtracheal wash specimens in comparison to cytospin and sediment smear preparations

Laura Haysom[1], Tekla M. Lee-Fowler[1], Elizabeth Spangler[2]
[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction: Storage and temperature have been demonstrated to significantly impact bronchoalveolar lavage fluid (BALF) analysis. Shipment of samples to a pathologist is often necessary, particularly those collected in private practices. Alternative cell preparation methods could limit storage and temperature effects. This study aimed to determine if Histogel and Gelfoam airway wash preparations were comparable to cytospin and sediment smear preparations.

Methods: Eleven bronchoalveolar lavage and 3 transtracheal wash samples were available for interpretation, including 8 canine, 1 feline and 5 equine samples. Cytospin and sediment smear preparations were created. Two milliliters of fluid was reserved for further analysis. Total nucleated cell count (TNCC) was determined via hemocytometer. The remaining fluid was used for Histogel and/or Gelfoam preparations. Each preparation was analyzed by a single board certified clinical pathologist and assigned a cellularity score (1-3) and a morphology score (1-4).

Results: Histogel and Gelfoam preparations resulted in poorer cellularity and morphology in comparison to cytospin preparations but did not differ significantly in comparison to sediment smear preparations. Cellularity scores for sediment smear, Histogel and Gelfoam preparations were inversely correlated with TNCC.

Conclusions: Cytospin preparations resulted in the best cellularity and morphology, and are therefore recommended whenever possible. Neither Histogel nor Gelfoam demonstrated any advantage over sediment smear preparations, and both performed poorly when compared to cytospins. Therefore, we do not recommend use of these methods. Total nucleated cell count impacts the cellularity of sediment smear, Histogel and Gelfoam preparations.

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Adenoviral Vectored Gonadotropin Releasing Hormone Vaccine for Estrus Suppression in the Mare

Rochelle L. Jensen[1], , Aime K. Johnson[1], Robyn R. Wilborn[1], ,Timothy D. Braden[2], Scot Roberts[3], Henry J. Baker[4]

[1]Department of Clinical Sciences, Auburn University, AL
[2]Department of Anatomy Physiology and Pharmacology, Auburn University, AL
[3] Vaxin Inc. Gaithersburg, MD
[4]Scott Ritchey Research Center, Auburn University, AL

Introduction. Estrus behavior of mares in training impedes performance and poses serious challenges for owners and trainers. Gonadotropin Releasing Hormone (GnRH) is the major reproductive hormone regulating estrus behavior and immunization against GnRH has proven successful in a number of species. Viral vectored vaccines can be engineered to express the GnRH decapeptide. The objective of this study was to evaluate the hormonal and behavioral effects of adenoviral vectored GnRH vaccination in mares, as measured by anti-GnRH antibodies, serum progesterone concentrations, and ovarian morphology via by transrectal ultrasound examination. Furthermore, the expression of estrus related behavior was evaluated by exposure to a stallion. It was hypothesized that immunized mares would not display behavioral signs of estrus and would not demonstrate cyclic fluctuations in progesterone concentration following a standard prime and boost vaccination protocol.

Methods. Nine non-pregnant cyclic mares were included in the study. Mares were assigned randomly to treatment (n=5) or control groups (n=4). Treatment group mares were vaccinated using a standard prime and boost vaccination protocol at weeks 0 and 4 using a dose of 1.5ml containing $1x10^{11}$ infectious particles of adenoviral (Ad5, E1/E3 deleted) vector, engineered to expresses GnRH antigen. Ovarian follicular activity was monitored twice weekly via transrectal palpation and ultrasound, and serum was collected weekly for progesterone and anti-GnRH antibody assays. Mares were also evaluated twice weekly for estrus behavior and assigned a score from 1 (diestrus) to 4 (estrus) based on behavior when exposed to a stallion.

Results. Four of five treatment mares continued to display normal cyclic behavioral estrus following vaccination as determined by a teaser stallion. One mare displayed an increased inter-estrus interval of 70 days, starting 7 days after her initial vaccination. During this time she did not display behavioral signs of estrus and serum progesterone concentrations were maintained above 2.2ng/ml. The remaining four mares experienced cyclical changes in serum progesterone concentrations ranging from <2.0ng/ml to 13.1ng/ml, were consistent with behavioral score for estrus, and were not different from that of control mares. Additionally, ovarian activity in treated mares was not different from control mares. Antibody data are not available at this time.

Conclusions. At the given antigenic dose and vaccination regime, the adenoviral vectored GnRH vaccine was not fully effective at inhibiting estrus related behavior in all treated mares. One mare exhibited a prolonged inter-estrus interval but appeared to remain in diestrus rather than an anestrous state expected to result from immunization against GnRH. Following evaluation of antibody data, further studies utilizing a larger antigenic dose and utilization of a larger sample size are needed to fully evaluate the efficacy of this vaccine in mares.

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Factors influencing environmental Salmonella incidence and proximal movement in a multi-species animal facility

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

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

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

Methods. Over a seven season period, 568 samples were collected from various large animal facilities and pastures within a veterinary college. Data was recorded to assess factors that contribute to increased incidence of *Salmonella* contamination. Samples were processed for *Salmonella* isolation according to published protocols, and *Salmonella* isolates were submitted to the National Veterinary Services Laboratories, Ames, IA, or Biovet, Inc., for serotyping. Data was analyzed with Statistical Analysis System (SAS).

Results. Of the 568 samples obtained, 203 (36%) samples were positive for at least one *Salmonella* serotype. *Salmonella* was recovered from the majority of facilities and areas sampled. Two cattle-associated serotypes, *S.* Muenster or *S.* Cerro, were isolated from most facilities regardless of residential animal species. Two cattle-associated serotypes, *S.* Muenster and *S.* Cerro, were isolated from multiple sites across multiple seasons. Over the course of the study, *S.* Cerro-positive facilities increased from 7% to 57%, which suggests movement of this serotype into adjacent animal facilities. The factors shown to be significant for isolation using Fisher's Exact Test for bivariate analysis were season ($p=0.008$), resident species ($p=0.008$), and environment ($p=0.05$). The highest number of *Salmonella*-containing samples was recovered during the warmer seasons and from areas exposed to dairy cattle. *Salmonella* isolates were recovered more frequently from man-made animal facilities compared to other variables.

Conclusions. The significantly increased frequency of *Salmonella* isolation from environmental samples exposed to dairy cattle indicates that this species is either the source of this pathogen, or is serving as an amplifying host for *Salmonella*. The recovery of cattle-associated serotypes supports this conclusion.

Acknowledgments. We thank Austin Conley, Heather Worley, Forrest Shirley, the Auburn University College of Veterinary Medicine J.T. Vaughan Large Animal Teaching Hospital, and various hospital section heads and administrators for their support and contributions to this research. This work was supported by the Animal Health and Disease Research Program of the College of Veterinary Medicine and the Alabama Agricultural Experiment Station.

Genomic Change of Bovine Viral Diarrhea Virus (BVDV) by Serial Infections in Sheep

T. Kuca[1], T. Passler[1], K.P. Riddell[2], J.D. Neill[4], B.W. Newcomer[1,2], H.L. Walz[3], P.K. Galik[2], Y. Zhang[2] and P.H. Walz[2]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[3]Thompson-Bishop-Sparks State Diagnostic Laboratory, Auburn, AL
[4]National Animal Disease Center, USDA Agricultural Research Service, Ames, IA

Introduction. BVDV isolates circulating in livestock herds show considerable variation in nucleotide sequence. Previous research in cattle demonstrated that most nucleotide changes resulting from the establishment of a persistent infection are introduced during the acute infection of the dam. As BVDV does not possess strict host specificity, infections in heterologous hosts (sheep, goats, camelids…) may provide further opportunity for generation of viral diversity. Using a BVDV isolate previously demonstrated to cause transplacental infection in cattle, goats, and white-tailed deer, this study investigated the changes introduced into the viral genome of BVDV during serial infections of pregnant sheep.

Methods. Six BVDV-naïve pregnant ewes between days 35 and 60 of gestation were included. On day 0, the first ewe was intravenously inoculated with 1 mL of media containing $1x10^6$ $CCID_{50}$ of BVDV-1b AU526. Every week one additional ewe was intravenously inoculated with 1 mL of BVDV-positive serum collected from the preceding ewe 5 or 7 days after inoculation. Blood samples were collected on days 5 and 7 after inoculation to detect viremia by virus isolation. Transabdominal ultrasonography and collection of blood samples were performed every 28 days until lambing to evaluate gestational viability and presence of antibodies to BVDV by virus neutralization, respectively. Blood samples were collected from the live lambs at the time of birth. Postmortem examinations were performed on aborted and stillborn fetuses, and dead lambs. Fetal tissues were collected and assayed for BVDV by virus isolation. Viral genome sequencing was ultimately performed on viruses obtained from all serum and tissue samples that were positive for BVDV by virus isolation.

Results. All six pregnant ewes were successfully infected with the BVDV-1b isolate AU526. All blood samples collected from the ewes on days 5 and 7 after inoculation were positive for BVDV by virus isolation. All ewes seroconverted to BVDV antibody positive by day 28 after inoculation. Two ewes aborted at 81 and 102 days of gestation, respectively. The remaining ewes delivered 3 live lambs, 2 stillborn fetuses and 1 fetal mummy. Two live lambs and two stillborn fetuses were found to be BVDV-positive at the time of delivery. Viral genome sequencing revealed up to 53 nucleotide changes introduced into the open reading frame of BVDV during the acute infection of the pregnant ewes. 49 to 56 additional changes were introduced during the establishment of persistent infection in the lambs. Interestingly, 5 to 11 nonsynonymous changes were detected at identical positions in the last five inoculated ewes and in two BVDV-positive lambs.

Conclusions. Transplacental infection with the BVDV-1b isolate AU526 was demonstrated in sheep as previously in cattle, goats, and white-tailed deer. Reproductive losses observed in pregnant sheep infected with BVDV were similar to previous reports. This study demonstrated the introduction of nucleotide changes into the genome of BVDV during serial infections in pregnant ewes. Unlike reported in cattle, many changes were similarly introduced during the acute infection of the ewes and the establishment of persistent infection in the lambs. The conserved nonsynonymous changes that were detected may be involved in host adaptation.

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Characterization and Simplification of a Bacteriophage Cocktail to Reduce Salmonella Lymph Node Carriage in Calves

J. Forrest Shirley[1], Steven R. Kitchens [1], Paul H. Walz[1], James C. Wright[1], and Stuart B. Price[1]

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

Introduction. Recent and ongoing research indicates that peripheral lymph nodes are important sources of pathogen contamination in ground beef. Previous findings in a model of Salmonella Enteritidis (SE) peripheral lymph node contamination in five to seven week-old calves indicated that seven, SE-targeting bacteriophages in a treatment cocktail are able to penetrate peripheral lymph nodes and are shed in appreciable numbers in the feces following oral administration. However, due to constraints involved in implementing a seven phage cocktail, characterization experiments were performed for each of the seven bacteriophage in the cocktail in order to establish exclusion criteria for cocktail simplification.

Methods. Electron photomicrographs were prepared by negatively staining phage with 2% phosphotungstic acid for and viewing with transmission electron microscopy. Qualitative lytic activity was assessed by performing Salmonella growth curves in the presence of bacteriophage (lysis curves) at varying multiplicities of infection. Additionally, Salmonella host range, efficiency of plating, and adsorption rate constants were determined for each cocktail phage.

Results. Cocktail phage were classified into three families based upon morphology: Myoviridae (three phage), Siphoviridae (two phage), and Podoviridae (two phage). Each cocktail phage demonstrated strong lytic activity against S. Enteritidis and were also able to kill and/or lyse a variety of Salmonella serovars. Cocktail phages also demonstrated lytic activity against a variety of Salmonella Serovars.

Conclusions. Except for cocktail phage in the Podoviridae family, lysis curve profiles, host ranges, efficiencies of plating, and adsorption rate constants clustered according to family, suggesting redundancy among cocktail phages in the Myoviridae and Siphoviridae families. Future work is will include determination of UV inactivation constants, RFLP, and lytic profiles of varying phage combinations to simplify and optimize the treatment cocktail. The long-term aim is a three-phage treatment cocktail designed to reduce Salmonella carriage in bovine peripheral lymph nodes.

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Characterization of nine novel naturally occurring melanocortin-4 receptor mutations

Wei Wang[1], Meng-Hong Dai[1,2], Xiu-Lei Mo[1], and Ya-Xiong Tao[1]
[1]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL
[2]Department of Basic Veterinary Medicine, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei, China

Introduction. The melanocortin-4 receptor (MC4R) is a member of family A rhodopsin-like G protein-coupled receptors (GPCRs). Extensive genetic studies suggested that MC4R is critically involved in regulating in food intake and energy expenditure. As the most common monogenic form of human obesity, about 170 naturally occurring *MC4R* mutations have been identified from different cohorts. Functional study of mutant MC4Rs is indispensable for proving the causal roles of *MC4R* mutation in obesity pathogenesis.

Methods. Human MC4R (hMC4R) mutants were generated by QuikChange™ site-directed mutagenesis kit. Stably transfected human embryonic kidney (HEK) 293 cells were used to investigate localization and quantification of hMC4R expression by confocal microscopy and flow cytometry, respectively. Transiently transfected HEK293T cells were used to detect ligand binding, cAMP signaling, and ERK1/2 signaling.

Results. In this study, we performed detailed functional characterization of nine novel human *MC4R* mutations (including L23R, K73R, T101N, T112K, M161T, L207V, M215L, R310K, and I316S) that have not been studied in detail before. Flow cytometry experiments showed almost all mutations had normal total expression compared with wild-type (WT) hMC4R. Five mutants, including T112K, M161T, M215L, R310K, and I316S, were expressed at similar levels as the WT hMC4R, while the other five mutants had decreased cell surface expression. Further confocal microscopy experiments were consistent with the flow cytometry results. Binding assay showed that four mutants had decreased maximal binding and no specific binding could be measured for M161T. Signaling assay showed that five mutants had either different Rmax or EC_{50}s in intracellular cAMP accumulation when stimulated with NDP-MSH or α-MSH. Three mutants had increased basal cAMP signaling. Western blotting experiments showed four mutants were defective in ERK1/2 signaling.

Conclusions. In summary, we provide detailed functional data for further studies on seeking novel therapeutic approaches for personalized medicine to treat obese patients harboring these *MC4R* mutations.

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Treatment with Endothelial Colony Forming Cells (ECFCs) and PEG-fibrinogen Encapsulated ECFCs Reduces Wound Surface Area in Equine Distal Limb Wounds

Randolph L. Winter[1], Wen J. Seeto[2], Fred J. Caldwell[1], Ashley N. Sharpe[1], Yuan Tian[2], David D. Pascoe[3], Jey W. Koehler[4], Elizabeth A. Lipke[2], and Anne A. Wooldridge[1]

[1]Department of Clinical Sciences, Auburn University, AL
[2]Department of Chemical Engineering, Auburn University, AL
[3]Department of Kinesiology, Auburn University, AL
[4]Department of Pathobiology, Auburn University, AL

Introduction. Endothelial colony forming cells (ECFCs) are progenitor cells which function in vascular repair and neovascularization. ECFCs may be useful therapeutically in conditions characterized by poor blood supply, such as distal limb wounds in the horse with exuberant granulation tissue (EGT). To ensure cell survival and site-specific localization with injection, ECFCs are encapsulated in the biomaterial poly(ethylene) glycol (PEG) coupled with fibrinogen. The introduction of ECFCs into equine distal limb wounds may promote enhanced neovascularization and therefore a more rapid healing process.

Methods. Three adult horses had two 6.25 cm^2 full-thickness dermal wounds created on each distal limb (8 wounds/horse). Each wound randomly received one of 4 treatments: serum; PEG-fibrinogen microspheres (MS); ECFCs alone; ECFCs encapsulated into MS (ECFC-MS). Four wounds (1 per treatment) were biopsied at baseline and then weekly, and four wounds (1 per treatment) were only biopsied at baseline and week 4. Wound healing was assessed weekly by wound surface area (WSA) analysis and granulation tissue scoring (GS) by blinded observers. Student's t-test, ANOVA, and a general linear model with pairwise comparisons were used for analysis with $P<0.05$.

Results. GS were greater (p=0.0039) for wounds biopsied weekly and for hindlimb wounds (p=<0.0001). GS was also different due to the individual horse (p=<0.0001). The effect of treatment group on the percent change in WSA was significant (p=0.0002), with ECFCs alone having the smallest WSA measurements. The ECFCs alone and ECFC-MS groups had significantly smaller WSA compared to MS alone (p=0.0005 and p=0.0014, respectively). Compared to serum, ECFCs alone had a WSA decrease which trended toward significance (p=0.0742).

Conclusions. Granulation tissue formation may be inherently greater in hindlimbs compared to forelimbs, which may dictate future study design. Additionally, weekly biopsies induced greater severity of granulation tissue. Injection of ECFCs alone or within PEG-fibrinogen microspheres significantly decreased wound size, which may indicate enhanced healing.

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Post-graduate/Faculty Platform Presentations

Successful Use of Stereotactic Image-Guided Biopsies in 5 dogs

Amanda R. Taylor[1], Brad Matz[1], P. Anthony Moore[1]

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

Introduction. The increased use of advanced imaging in veterinary medicine has led to frequent identification of disease in locations difficult to access. These include lesions in the brain, caudal nasal passages, retrobulbar space or paraverterbral tissues which have required invasive methods for biopsy. Diagnostic yield from these invasive approaches may be as low as 61% for nasal biopsies. A novel approach using stereotactic, image-guided technology allows for minimally invasive approaches with successful results in diagnosis.

Methods. Client-owned dogs were placed under general anesthesia and fiducial markers were secured. Dogs were imaged in 3 planes with MRI or CT. Fiducialized images were imported into the Brainsight™ workstation. Once imported, a referenced image reconstruction was created. Infrared cameras were used to correlate the biopsy instrument position with the dog and the lesion to be biopsied. Biopsied tissue was evaluated with cytology and histopathology. After the procedure the patients were followed for adverse events.

Results. Five dogs underwent this procedure for biopsy of nasal masses (3), a retrobulbar mass (1) and a vertebral mass (1). A diagnosis was achieved in all 5 patients, with a diagnostic yield of 100%. One patient was unable to be registered due to fiducial movement. No adverse events secondary to the use of stereotaxy were reported.

Conclusions. The stereotactic equipment has high accuracy in targeting lesions within the caudal nasal cavity, retrobulbar space and vertebral spinous processes. Movement of fiducial markers prior to the procedure results in inability to perform the procedure sterotactically.

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Undergraduate Poster Presentations

Evaluation of In Vitro Cross-neutralization between Bovine Parainfluenza 3 Virus Genotypes

Jessica Isaac[1], Yijing Zhang[1], Kay P. Riddell[1], Patricia K. Galik[1], Paul H. Walz[1], and Benjamin W. Newcomer[1]

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

Introduction. Bovine parainfluenza 3 virus (BPI3V) is in the family Paramyxoviridae and genus Respirovirus and exists as three distinct genotypes (A, B, and C). Clinical signs of infection include cough, fever and moderate nasal and ocular discharge; infection may predispose cattle to bovine respiratory disease complex (BRDC). Historically, only BPI3Va has been present in the US but recently, both non-A genotypes have been isolated from diseased American cattle. However, current vaccines contain only BPI3Va strains. Therefore, the objective of this study was to analyze the degree of cross-neutralization between the various genotypes of BPI3V.

Methods. For this study, the virus neutralization (VN) test was used to analyze the efficacy of cross-neutralization between BPI3V antibodies. The tests were performed using samples from each BPI3V genotype, four subgenotypes of BPI3Va, two subgenotypes of BPI3Vb, and the lone subgenotype of BPI3Vc; monospecific antisera from each genotype were assayed against a viral isolate from each BPI3V genotype and subgenotype by comparing their neutralization titers. The VN testing was performed on 96-well plates in triplicate. Titers were calculated by observing the presence of cytopathic effect in each assay.

Results. The serum from a BPI3Va strain was the most effective against A- genotypes and subgenotypes as evidenced by higher titers in the VN assay compared to the non-A isolates. However, the results of the VN assays assessing the efficacy of BPI3Vb and –c antibodies were less definite. Sera containing antibodies to BPI3Vb or BPI3Vc were not markedly more efficient at neutralizing homologous isolates than heterologous isolates as measured by observed VN titers.

Conclusions. Monospecific antibodies to BPI3Va are most effective against BPI3Va isolates while antibodies against the non-A genotypes appear to neutralize both heterologous and homologous isolates relatively equally. Therefore, antibodies generated from current BPI3V vaccines may not be effective against all the BPI3V strains. More research related to evidence of cross-neutralization should be done to confirm the results of this study and evaluate the effectiveness of current BPI3V vaccines.

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Pathogen Associated Molecular Pattern (PAMP) Receptor Expression in Chickens Before and After IBV Challenge

Isabelle Kallenberg, Stephen L. Gulley, Frederik W. van Ginkel

Scott-Ritchey Research Center, Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL

Introduction. Infectious Bronchitis Virus (IBV), a highly infectious single-stranded positive sense coronavirus, is one of the leading causes of economic loss within the poultry industry. Although attenuated and inactivated vaccines are extensively used to control IBV, IBV infections persist in chicken flocks. In non-vaccinated flocks, a 100% morbidity rate has been observed. One potential contributing factor to the lack of vaccine-mediated immune protection may be the immunologically immature state of the chicks when vaccinated. The immune response is initiated in the host by activating pathogen associated molecular pattern (PAMP) receptors when infected by a virus such as IBV. Therefore we hypothesize that PAMP receptors are important in the control of IBV in chickens and are expressed at lower levels in young chicks.

Methods. Spleen, conjunctiva-associated lymphoid tissue (CALT), and Harderian Glands (HDGL) were collected from specific pathogen-free white leghorns from Sunrise Farms in NY at 3 weeks and 2 days of age. The leghorns were challenged with IBV field strain AL4614-98. The tissues were homogenized using Next-Advanced Bullet Blender with 1 mm diameter zirconium oxide beads in Tri-Reagent. RNA was isolated according manufacturers protocol and normalized to 50 ng/µL using a Nanodrop Spectrophotometer. The PCR reaction was performed using Q-Script 1 Step SYBR green master mix. Quantitative-Reverse Transcription PCR was performed on the BIORAD CFX-96 for 40 cycles using previously published primers (Vervelde et al., 2012; Lowenthal et al., 2011; Jie et al., 2013; Li et al., 2005).

Results. In 3-week-old controls chickens, the mRNA expression of TLR-3, TLR-7, and MDA5 was significantly higher in CALT and HDGL compared to the spleen, while expression of TLR-15 and TLR-21 did not significantly differ in these tissues. Expression of PAMP receptors in CALT and HDGL was very similar and only differed significantly for TLR-7. Following IBV-challenge MDA5, TLR-3, TLR-7, TLR-15, TLR-21 expression were significantly higher in 3 week old chickens between 1 and 4 days after ocular IBV challenge in HDGL and/or CALT than the control group. Comparing 3-week-old to 2-day-old chickens, significant differences in MDA5, TLR-7, TLR-15, TLR-21 expression were observed in magnitude and/or kinetics in CALT. There was also a significant difference in TLR-7 expression level in HDGL.

Conclusions. In the control group, higher gene expression of PAMP receptors in mucosal tissues, such as CALT and HDGL, was observed, indicating that PAMP receptors could play an important role in the first line response to IBV in chickens. Following ocular IBV challenge, 3-week-old chickens had higher levels of expression compared to 2-day-old chicks for MDA5 and TLR-7 in CALT, and TLR-7 in HDGL. The data supports our hypothesis. After IBV challenge an early (days 1 and 2) and late (days 3 and 4) response in PAMP receptor expression were observed in mucosal tissue. In the spleen, limited differences in PAMP gene expression were observed upon IBV challenge. This confirms the importance of mucosa-associated lymphoid tissues in initiating an adaptive immune response to IBV. The lack of PAMP receptors may explain the diminished immune responses to IBV in young chicks.

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Veterinary Student Poster Presentations

Circulating Plasma miRNAs as Novel Biomarkers in Congestive Heart Failure

Kelsi Anderson, Amy Bohan, and SeungWoo Jung

Auburn University CVM, Auburn, AL

Introduction. Myxomatous mitral valve disease (MMVD) is the most prevalent canine heart disease. Congestive heart failure (CHF) carries poor prognosis and often leads to cardiac death in dogs with MMVD. Early identification of dogs prone to developing CHF is critical to improve long term survival outcome. The aim of this study is to characterize molecular profiles in dogs with CHF secondary to MMVD and to develop novel plasma molecular biomarkers via liquid biopsy. MicroRNAs (miRNAs) are small, non-coding RNAs and have been known to regulate expression levels of genes involved in human CHF. The hypothesis tested in the study was that plasma miRNAs may be differentially expressed in dogs with CHF secondary to MMVD.

Methods. A minimum of seven dogs in each group (normal vs CHF) were needed to demonstrate the statistical power with a probability of 0.9 and type one error of $p \leq 0.05$ based on previous publications. Blood was collected in EDTA tubes from 7 normal geriatric dogs (free of heart murmur and absent history of CHF) and 7 dogs with CHF secondary to MMVD (confirmed by clinical signs, chest x-ray and echocardiography). Plasma samples were subjected to isolation of miRNAs with a miRNeasy Plasma Kit (Qiagen) and subsequently reverse-transcription reaction with a miScript II RT Kit (Qiagen). Validated canine primers of 10 candidate miRNAs and SNORD (housekeeping gene) were employed to quantify relative expression levels of each miRNA between normal and CHF dogs via real time PCR (miScript SYBR Green PCR Kit, Qiagen).

Results. Canine specific miRNAs were successfully isolated from plasma and amplified. Expression levels of miR-21, involved in cardiac hypertrophy and fibrosis, increased in CHF dogs with statistical significance (normal mean: 0.49 vs CHF mean: 1.12; p-value of 0.015) when compared to normal dogs. These results suggest that altered miRNAs expression profiles may play a role in cardiac progression to CHF in dogs with MMVD.

Conclusions. Further investigation remains warranted to determine their diagnostic potential as molecular biomarkers for early detection of CHF in dogs with MMVD.

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Validation and Genetic Profiling of Canine Mammary Tumor Receptor Gene Expression

Jordan C. Ayers DVM Candidate, Dr. Richard Curtis Bird PhD, Farruk M Lutful Kabir PhD Fellow, Patricia DeInocentes , and Dr. Pamela Ehrenfeld PhD
College of Veterinary Medicine Auburn University, AL 36849

Introduction. This project used conserved primers to create a profile of the expression of six genes associated with both human breast cancer and canine mammary tumors. By validating, quantitation and PCR assays we have demonstrated that these genes are expressed in both cancers, we now have a basic genetic profile by which to characterize these tumors. This was accomplished in 9 established CMT cell lines.
In the next phase of our research, we used primers for these conserved genes with freshly obtained tumors to create a profile and provide insights into the phenotypes of individual patient tumors. Expansion of this profile and the further validation of the expression patterns have huge implications, not only in veterinary medicine, but in human medicine as well. This objective is currently being completed with three of the six genes having gone through validation on 12 cell lines obtained from masses removed from routine surgical removal of mammary tumors.
By creating a gene expression profile for CMTs allied to neoplastic transformation, tumors can be more precisely phenotyped so veterinarians can know the most effective courses of treatment and associated prognoses. Identifying the gene expression patterns associated with CMTs allows for a better understanding of malignant behavior of CMTs; this not only has huge implications in management of disease but also in the development of treatments to better combat this highly aggressive form of cancer. Currently, treatment protocols for CMT's are universal among canines. Most other domestic species have monoclonal mammary tumors that do not vary much, but for the canine a universal protocol is not effective. As is the case in human breast cancer, the CMT is a hormone dependent tumor and by targeting that specific tumor type treatments can be more effective and, possibly, have fewer or less severe side effects.
Also, this study opens the possibility of further comparison of canine and human breast cancer geno- and phenotypes, by looking at common mutations associated with human breast cancer as well as in CMT. Validation of canine disease as a model system for breast cancer in women will allow development and testing of new treatments for the benefit of both canine and human patients. It will also promote the translation of treatments between species promoting better disease management in human and canine patients.

Methods. Primer design; How to design and run a PCR (polymerase chain reaction); Cell culture; RNA extraction; Determining quality and quantity present for isolated RNA; Gel extraction and preparing DNA for sequencing ; Pouring gels and tailoring said gels to the size of the amplicon

Results/Conclusions. Each tumor cell line showed a unique expression pattern, indicating genetic profiling of CMT's are just as necessary as with breast cancer
This study is ongoing, as the fresh cell lines are incompletely validated and there are several more cell lines being examined. However, these initial results show that the genes are expressing in the novel lines and that the expression is different between lines. This indicates unique expression patterns on a patient by patient basis, similar to breast cancer genes in women.
It appears that common defects seen with the neoplastic transformation of mammary tumors are similar to those seen in the canine model
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Monitoring Humoral Immune Response to Viral Vector and Osteosarcoma Tumor Antigen

Elizabeth Gammon[1], Payal Agarwal[2], and Bruce F. Smith[2,3]
[1]College of Veterinary Medicine, Auburn University, AL
[2]Scott-Ritchey Research Center, Auburn, AL
[3]Auburn University Research Initiative in Cancer, AL

Osteosarcoma is an aggressive bone cancer often affecting distal extremities in large breed dogs. Amputation of the affected limb is standard therapy to alleviate the pain but amputation alone still results in a median survival time of only about 6 months, with 90% of deaths occurring due to pulmonary metastasis. When chemotherapy is added to therapy the median survival extends to 1 year but metastasis inevitably results. The metastasis of this cancer needs to be addressed differently in order to improve survival times. Oncolytic virotherapy presents a potential means to target the cancer as it spreads through the body. A conditionally replicative adenovirus (CRAd), engineered with an osteocalcin promoter driving the E1 gene (necessary for replication) will facilitate replication of the adenovirus only in cells expressing the osteocalcin promoter. This project also addresses the role of the patient's own immune response in this process, specifically the humoral immune response. The dogs in this clinical trial had all been previously vaccinated with canine-adenovirus 2, a core vaccine, and the CRAd administered replicates, expresses CAV-2 antigen in infected cells and potentially incites existing immunity in the dog to kill cells with this viral antigen. The virus may eventually stimulate an immune response additionally against tumor cells in recognition of tumor antigen. This would potentially allow long-term protection with activated immunity against tumor cells, even those not infected with the virus. Clinical trial dogs were observed for antibody production in a comparison between blood samples collected pre and post-exposure to the CRAd. Primary cell cultures from the dog's primary tumor were exposed to primary antibody, autologous serum from the blood samples, and then labeled with secondary antibody goat anti-canine to measure using flow cytometry. Antibody was increased in patient serum in the post-exposure samples relative to their pre-exposure samples in 4 of the 7 dogs tested.

Redox-Responsive MRI Contrast Agents for the Detection of Doxorubicin-Induced Oxidative Stress in the Heart.

Megan L. Hataway [1], Cristine Robinson [1], Russell Cattley[2], Cynthia Hutchinson [2], Ron Beyers [3] and Dean Schwartz [1]

[1]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathobiology, Auburn University, AL
[3]Auburn University MRI Research Center, Auburn, AL

Introduction. Oxidative stress is thought to be a contributing factor to the progression of many cardiovascular diseases. Oxidative stress is a general term used to describe the steady state level of oxidative damage in a cell or tissue caused by reactive oxygen species (ROS). Oxidative stress results when the body's anti-oxidative defenses cannot keep levels of ROS sufficiently low. Doxorubicin (DOX) is one of the most effective anticancer drugs to treat a wide variety of cancers such as lymphoma, sarcoma, breast cancer, and pediatric leukemia. The long term use of doxorubicin is limited, however, due to the well documented cumulative dose-dependent irreversible cardiotoxicity. Although the underlying cause of ROS is unknown, the increase in myocardial ROS is one of the underlying causes for DOX-induced cardiotoxicity. Our group has shown that the redox-responsive MRI contrast agent, H_4qtp2, increases contrast in the hearts of doxorubicin treated rats. The goal of this project was to determine the level of oxidative stress in these hearts.

Methods. Rats were treated with DOX (15 mg/kg) for 72 hours. Rats were anesthetized with isoflurane and monitored by electrocardiogram. H_4qtp2 was infused intravenously at 10 mg/kg and then the hearts were sectioned for ex-vivo MRI scanning and frozen in liquid nitrogen. On the frozen tissue, TBARS assay was performed using malondialdehyde as a standard for lipid peroxidation. Western blotting for cleaved Poly ADP ribose polymerase (PARP) was used as a measure of apoptosis and real time PCR was used to measure expression of oxidative stress genes.

Results. The concentration of MDA did not change between vehicle and DOX treated rats suggesting DOX treatment did not produce lipid peroxidation at 72 hours of treatment. PARP cleavage in vehicle and DOX treated hearts also showed no difference suggesting that the apoptotic pathway has not been turned on in DOX treated rats. Redox responsive antioxidant genes were evaluated using RT-PCR. Gene expression for glutathione S-transferase 1 (GSTA-1), hemeoxygenase-1 (HO1), microsomal S-transferase 1 (MGST1), and NAD(P)H dehydrogenase quinone 1 (NQO1) were all enhanced in the hearts of DOX compared to vehicle-treated rats.

Conclusions. The redox-responsive MRI contrast agent, H_4qtp2, can detect changes in redox status in the heart of DOX treated rats before histological or overt signs of oxidative stress are observed.

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Friction Measurements of Intact Equine Carpal Articular Cartilage

Lyndsey R Hayden[1], R. Reid Hanson[1], Patrick Jones[1], Gabby Willhelm[2], Dewey R. Wilhite[1], Robert Jackson[3]
[1]Department of Clinical Sciences, Auburn University College of Veterinary Medicine, Auburn, AL
[2]Department of Animal Science, Auburn University, Auburn, AL
[3]Department of Mechanical Engineering, Auburn University, Auburn, AL

The goal of this experiment was to develop a novel, robust method of cartilage on cartilage friction testing. This method will determine the coefficient of friction (COF) of the migrating contact area when the proximal articular surface of the second carpal bone is brought into contact with the articular cartilage of the distal radial facet. This method allows for a more accurate determination of the COF, as it more closely resembles the physiological properties in vivo. Samples were divided into two groups, (fresh and frozen) and two locations (axial and abaxial) on the distal radial facet. Three different testing parameters were designed to observe the friction under a migrating contact area: 5N for 5 min (test 1), 10N for 5 min (test 2), and 10N for 42 min (test 3). This method of cartilage on cartilage friction testing sustained a low COF when a normal load was applied to a migrating contact area under these testing parameters, 5N for 5 min, 10N for 5 min, and 10N for 42 min.. No significant difference was observed overall when comparing the fresh and frozen groups, as well as comparing the two different locations tested on the distal radial facet. It was determined that there is no significant increase in the COF with an increase in load and time; however, it is shown that there is a statistical difference when comparing two different loads in the fresh. These results show that this novel method of testing is an accurate method of determining the friction coefficient of a migrating contact area.

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Identification and characterization of Glanzmann Thrombasthenia in a beagle-mix dog

Rebecca Kennerly[1], Reka Muller[1], Laura Haysom[2], Peter Christopherson[1], Kevin King[1], Mary Boudreaux[1]

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

Introduction. Glanzmann thrombasthenia (GT), an inherited intrinsic platelet disorder, results from a quantitative or qualitative defect of the platelet membrane glycoprotein complex IIb-IIIa (GPIIb-IIIa). The GPIIb-IIIa complex is also known as integrin αIIb-β3 and as the fibrinogen receptor and is the most abundant receptor on the platelet membrane. The GPIIb-IIIa complex mediates normal platelet aggregation; patients with GT have abnormal hemorrhage secondary to impaired platelet function. GPIIb and GPIIIa are encoded by separate genes and mutations in either gene can result in GT. Over 500 causative mutations for GT have been identified in people. In veterinary medicine GT has been documented in Otterhounds, Great Pyrenees, and in several horse breeds. All of the mutations identified in animals have been in the gene encoding GPIIb. This report describes a 2 year old, intact, female beagle-mix dog obtained by the owner as a stray that had a life-long history of petechial hemorrhages

Methods. The patient's coagulation screening test results, platelet counts, and von Willebrand factor antigen levels were normal. A buccal mucosal bleeding time and clot retraction test were performed; platelet aggregation and flow cytometry experiments were performed using platelet-rich plasma isolated from the patient and a clinically normal control dog. DNA was isolated from whole blood and subjected to PCR to amplify the coding regions of the genes encoding GPIIb and GP IIIa in the patient.

Results. The buccal mucosal bleeding time was prolonged at > 5 minutes. Platelets responded with shape change but failed to aggregate in response to all agonists tested (ADP, collagen, PAF, and gamma thrombin). Clot retraction failed to occur. GPIIb and GPIIIa were absent from platelet membranes as assessed by flow cytometry. All coding regions and exon-intron splice sites of the genes encoding GPIIb and GPIIIa were evaluated. A single nucleotide polymorphism (SNP) at position 1264 (C1264T) in the gene encoding GPIIb was identified. This change results in a premature termination codon and is predicted to change the arginine encoded at position 422 to stop (R422X). Heterozygosity was not noted in any of the regions evaluated suggesting the sire and dam were closely related.

Conclusions. This is the first documentation of GT at the functional, biochemical, and molecular level in a beagle-mix dog. It is likely that Daisy's sire and dam were closely related as an explanation for the presence of a single mutation causing GT in a mixed breed dog.

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Search for a Pan-Tumor Promoter for Use in Conditionally Replicative Adenoviral Vectors

Samantha Morici, Maninder Sandey, Payal Agarwal, Richard Rathbun, Elizabeth Yanchak Gammon, and Bruce F. Smith

Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL

Introduction.
Conditionally Replicative Adenoviruses (CRADs) are genetically modified viruses that can be used in the treatment of cancer. Their functionality resides in the fact that they are tumor-specific, having only the capability of being internalized by and replicating in tumor cells. The key to this tumor-specificity is the promoter, a section on the DNA of the virus that controls the replication of the viral genome. The exogenous promoter Progression Elevated Gene 3 (PEG3) from rats has not only shown tumor-specificity in the human model, but has also shown pan-tumor properties - having the capability of replicating in almost all tumor cells. Regardless of its success, PEG3 has not been tested for tumor-specific or pan-tumor properties in the canine model. Three other promoters, CXCR4, TERT, and Survivin have shown similar pan-tumor expression properties in human and murine models but these endogenous canine promoters have not been evaluated.

Methods.
In order to explore the tumor specific activity of PEG3, the rat promoter was joined with a GFP reporter gene and was evaluated using a series of cellular transfections and infections which were compared with a CMV-driven GFP control. Endogenous canine promoters CXCR4, TERT, and Survivin were examined using Reverse Transcriptase qPCR and compared to a Beta Actin positive control.

Results.
Results showed PEG3 expression in CML10, CMT28, FDK1, and MDCK cell lines. cTERT also showed negligible expression differences between normal and tumorous cells whereas cSurvivin and cCXCR4 showed markedly higher expression in tumor cells when compared with normal tissues and normalized against a beta actin positive control.

Conclusions.
A series of transfections, infections, and flow cytometric analyses show that PEG3 is not tumor-specific. Likewise, amplification of cTERT in a broad range of both cancer and normal tissues using qRT-PCR shows that cTERT is not tumor-specific. Analysis of cCXCR4 and cSurvivin shows markedly enhanced expression in tumor cells when compared with normal tissues, warranting further study of theses promoters.

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Evaluation Of An Aqueous Extract Of Terminalia Chebula For Anti-Arthritic Efficacy And Safety In Osteoarthritic Dogs: Radiographic Evidence

Nichole Murdock[1], Ramesh C. Gupta[1], Natalia Vega[1], Katie Kotora[1], Jean Miller[1], John T. Goad[1], Michelle A. Lasher[1], Terry D. Canerdy[1], Sanyasi R. Kalidindi[2]
[1]Murray State University, Murray/Hopkinsville, KY, USA
[2]Natreon Inc, New Brunswick, NJ, USA

Introduction. Currently, ~ 20% of the adult and 80% of geriatric dog population in the US (>80 million) suffer from osteoarthritis (OA). The present investigation was carried out to assess anti-inflammatory and anti-arthritic properties and safety of a standardized aqueous extract of *T. chebula* (TCE) in moderately OA dogs.

Methods. Dogs with OA received either 500 mg placebo or 500 mg TCE b.i.d. for 150 days. Each month, dogs were given a full physical exam and were evaluated for arthritic pain using different criteria, joint flexibility using goniometer, CBC and erythrocyte sedimentation rate (ESR), and serum biomarkers of liver (bilirubin, ALT, and AST), kidney (BUN and creatinine), and heart and skeletal muscle (CK) functions. Elbow and stifle joints were radiographed on day 0 and day 150 for evaluation of osteophyte formation and cartilage damage.

Results. Dogs given TCE showed significant ($P<0.01$) reductions in overall pain, pain upon limb manipulation, and pain after physical exertion by 60-90 days, with maximum effect on day 150. A marked reduction in ESR coincided with pain reduction in treated dogs, which was indicative of anti-inflammatory effect of TCE. Radiographic evidence also indicated a marked decrease in osteophyte formation and cartilage damage. No significant change occurred in physical parameters, serum biomarkers or CBC parameters in dogs on placebo or treatment, which suggested that TCE was well tolerated.

Conclusion. It can be concluded that *T. chebula* extract, by having many active principles, such as chebulagic acid, chebulinic acid and low molecular weight hydrolysable tannoids, might have provided anti-inflammatory and anti-arthritic effects in dogs without causing any side effects.

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The Impact of Analgesic and Anesthetic Drugs on Sperm Motility in Equine and Bovine Species

Jaida Reeves[1], Jack Kottwitz[1] DVM, Robyn Wilborn[2] DVM, MS, DACT, Misty Edmondson[2] DVM, MS, DACT and Dawn Boothe[1] DVM PhD DACVIM DACVCP

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

Introduction. Human research indicates that certain drugs, specifically opioid class drugs, have a profound adverse effect on sperm cell motility. It is theorized that the drugs used for anesthesia and analgesia in veterinary medicine have a similar negative effect on sperm motility in veterinary patients.

Methods. Semen was collected from stallions utilizing an artificial vagina and from bulls by electro-ejaculation. Semen concentrations were standardized to at least 60 million sperm cells per ml in Ham's F10 Cell Culture Media. The following drugs were evaluated for effects on sperm motility: Butorphanol (μ and κ opioid partial agonist/antagonist), Naloxone (opioid antagonist), Xylazine (alpha-2 agonist) and Detomidine (alpha-2 agonist). All drugs were examined at concentrations of 0.01 μg/ml, 2.00 μg/ml, 25 μg/ml, 50 μg/ml, and 100 μg/ml. In addition, drug concentrations of 300 μg/ml and 400 μg/ml were evaluated in bulls. Computer-assisted sperm analysis (CASA) was utilized to determine sperm motility at the time of drug exposure and at specific time intervals set individually for each species. Sperm motility was assessed as percent progressive motility. Results were evaluated by one-way analysis of variance and unpaired t-test to identify significant differences between controls and individual drug concentrations. The level of statistical significance was set at $P < 0.05$.

Results. In stallions butorphanol induced a significant increase in progressive sperm motility at dose concentrations of 0.01 μg/ml, as well as a significant decrease in motility at or above dose concentrations of 25μg/ml, when compared to controls. Detomidine had significant negative effects at drug concentrations of 0.01 μg/ml, and at or above 25μg/ml. Xylazine produced a significant negative impact at the drug concentration of 100μg/ml. In bulls butorphanol caused a significant decrease in progressive sperm motility when compared to controls with dose concentrations at or above 100μg/ml. Detomidine had significant negative effects at dose concentrations at or above 100μg/ml along with a total cessation of progressive motility at or above 300μg/ml. Xylazine had a statistically significant negative impact with dose concentrations at or above 300μg/ml. Naloxone did not significantly alter progressive sperm motility in bulls or stallions at all drug concentrations evaluated. The drug preservatives methylparaban and benzothonium chloride were evaluated at the doses present in the drug concentrations used with no effect on sperm motility.

Conclusions. In stallions and bulls the data shows a negative impact on sperm motility from exposure to butorphanol, detomidine, and xylazine *in vitro*.

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The Pharmacokinetics of Altrenogest (Regu-Mate®) in Lactating Mares and Suckling Foals

Charles R. Rehm[1], Robyn R. Wilborn[1], Dawn M. Boothe[2], Crisanta Cruz-Espindola[2], Aime K. Johnson[1], Natalie S. Fraser[1], D. Andrew Hestad[1], Erin S. Groover[1]
[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]Department of Clinical Pharmacology, College of Veterinary Medicine, Auburn University, AL

Introduction: Altrenogest is a synthetic progestin that has been used routinely in mares for over twenty years for the purpose of decreasing estrus behavior in performance mares, synchronizing the estrus cycle in broodmares and maintaining difficult pregnancies in high risk broodmares. The objective of this research is to determine if altrenogest (Regu-Mate®) is inadvertently passed from treated mares to their suckling foals via lactational transfer. This data would then determine if further investigations are warranted to examine the physiological effects, primarily reproductive and anabolic, of this drug on foals.

Methods: Quantitative drug concentrations in mare plasma, mare milk, and foal plasma were measured via liquid chromatography-tandem mass spectrometry through the Auburn University Clinical Pharmacology Lab. To optimize detection as well as establish the pharmacokinetics of altrenogest, blood and milk samples were taken at time 0, 15 min, 30 min, 45 min, 60 min, 90 min, 2 hr, 3 hr, 4 hr, 6 hr, 8 hr, 12 hr, 18 hr, and then every 6 hours through 72 hours for each mare-foal pair. Samples were cryogenically preserved at -80 degrees Celsius until testing. Unfrozen time 0 milk was also sent to Mid-South Dairy Records for a compositional screening analysis of fat, protein and somatic cell count. Both sampling phases were conducted when the foals were six weeks of age. Mares (n=9) were orally administered a standard dose of altrenogest (0.044mg/kg SID for 3 days) during phase one. During phase two, mares (n=4) received a double dose of altrenogest (0.088mg/kg PO SID for 3 days). Foal urine was also collected via free catch or urinary catheterization during phase two at times 0, 8, and 24 hours after drug dosing to determine whether altrenogest was detectable in the urine.

Results: In phase one (standard dose), altrenogest was detected and quantified in mare plasma, detected in mare milk, but was not detected in foal plasma. The standardized milk analysis revealed no correlation between milk protein/fat composition and pharmacokinetic data. Results of phase two (double dose) are pending.

Conclusion: Detection of altrenogest in mare plasma during phase one confirms that altrenogest was administered properly and is orally bioavailable to the mare. The detection of drug in the mare's milk confirms for the first time that the hormone is excreted in this manner and indicates that the foal is exposed to altrenogest via suckling. However, the bioavailability of altrenogest to the foal has not been established. Pending results from phase two are necessary to determine if altrenogest is detectable in foal plasma or urine in order to establish successful lactational transfer of this hormone.

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Thru-Hiking the Appalachian Trail: The Effect of Motivation and Personality on Successfully Accomplishing Long-Term Goal

Roemhild, Camille E.

Department of Psychology, Sweet Briar College

Introduction: Long-term goals must be approached differently than short-term goals because the final reward and payoff for endurance and hard work is delayed. Previous research has found that motivational factors and personality traits influence the level of success people have in accomplishing such goals. In achievement goal theory, the focus is on the reasons people pursue a task, rather than what the goal is and how they accomplish it (Wigfield et al., 2006). Grit, another type of motivation, examines ongoing work towards goals, persistence through failures and adversity, and stamina (Duckworth, Peterson, Matthews, & Kelly, 2007). Conscientiousness correlates with Grit and entails thoroughness, reliability, organization, self-control, and industriousness. The Big Five model does not differentiate between achievement-orientated people and dependable people in the conscientiousness scale (Hough, 1992; Duckworth et al., 2007). This study explored the relationship of motivation and personality on successfully accomplishing long-term goals in a natural setting with participants who had chosen the same goal voluntarily.

Method: Thru-hikers were surveyed on the trail between NC and GA. Thirty-one males and 17 females ranging in age from late-teens to early-sixties were initially given the NEO Five-Factor Inventory-3 and a survey based upon Goal-Setting Theory, Achievement Motivation, and Grit compiled by modifying or copying questions from previous research. Fourteen females and 22 males completed the post-test, which asked how much of the trail they had completed, along with questions about their experiences while hiking.

Results and Conclusion: Both *t*-tests and correlations show partial support for my hypotheses. One facet of Grit, perseverance of effort, did not differ between those who did or did not complete the thru-hike; however, the second facet of Grit, consistency of interests, was significant, $t(34)=2.822$, $p=.008$, with those who completed hiking the trail scoring higher. This shows that being able to stay focused on one thing leads to higher success in achieving a long-term goal. Tenacity, which combines effort and persistence, was significantly positively correlated with the percent of the trail completed, $r(34)=.375$, $p=.024$. Those who completed the trail scored significantly higher in conscientiousness, $t(34)=2.175$, $p=.037$. Conscientiousness was positively correlated with perseverance of effort and consistency of interests, $r(34)=.706$, $p<001$; $r(34)=.425$, $p=.009$. Those who scored high in mastery avoidance (a maladaptive orientation) were significantly less likely to complete the thru-hike, $t(34)=3.003$, $p=.005$. Disorganization, which is negatively associated with performance, was marginally significantly related to completing the trail, $t(34)=1.992$, $p=.054$. Those scoring higher in disorganization were less likely to complete the trail than those with a low score. These results show those able to maintain drive and interest, keep organized, and not focus on the negative were more likely to accomplish a long-term goal.

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Mitophagy and Histone Deacetylase Inhibitory Activity of Small Molecule Carnitinoid Antioxidant Compounds

Kathleen R. Stewart, Michelle Hoffman, and Michael H. Irwin*
Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
*Corresponding author

Introduction. Perturbations in the process of mitophagy (mitochondrial recycling, a specialized form of autophagy) are thought to contribute to the pathogenesis of Parkinson's disease (PD) and certain other mitochondrial dysfunctions. Beclin-1 is a pro-autophagy protein, whose production is upregulated during periods of increased mitophagy. Another promising target pathway is the inhibition of histone deacetylase (HDAC) activity by carnitinoid compounds. HDAC inhibition influences gene expression by upregulating a subset of genes, including genes involved in the antioxidant response element (RSE) and others that may have anti-cancer properties. Butyrate inhibition of HDAC activity has been found to enhance memory recovery and promote neurogenesis after ischemic insult. Small molecule antioxidant carnitinoid compounds (lipoyl- and butyrylcarnitines) have been found to have a neuroprotective effect during periods of mitochondrial insult, and may have both HDAC inhibitory activity and pro-mitophagy activity.

Methods. Several novel carnitinoid compounds were investigated for their ability to promote upregulation of Beclin-1 expression and to inhibit HDAC activity in cultured cells. Rotenone (a mitochondrial complex I inhibitor that induces a PD-like syndrome in rodent models) was used to induce mitochondrial dysfunction in rat H19-7/IGF-IR primordial hippocampal neurons *in vitro*. Cultures were co-treated with four distinct carnitinoid compounds to determine mechanisms for their observed protective effect on neurons. Beclin-1 expression was assessed by western blotting. Inhibition of HDAC activity was assessed by western blotting of acetyl residues on histone protein H3 and by a live cell plate-based HDAC activity assay.

Results. Western blot analysis to measure Beclin-1 expression (balanced against β-actin, a constitutively expressed protein found in all cells) showed no significant differences among the different treatments. Further investigation is warranted to determine the potential involvement of other proteins involved in mitophagy. All four PMX compounds showed measurable HDAC inhibitory activity compared to control (untreated) cells in the live cell plate-based assay and by western blotting of acetylated histone H3 protein.

Conclusions. PMX lipoylcarnitine and butyrylcarnitine compounds, with demonstrated neuroprotective effects in rat models of mitochondrial dysfunction, showed HDAC inhibitory activity *in vitro*. Further studies are currently underway to determine the mechanisms involved in neuroprotection by these novel carnitinoid compounds and their potential to ameliorate or reverse PD-like clinical signs *in vivo* using rat and mouse models of mitochondrial dysfunction.

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In Vitro Sensitivity of Canine Glioblastoma Cells to Temozolomide

Emily D. Velez[1], Sofia A. Castello[2], Anna C. Robinson[2], Jennifer (Jey) W. Koehler[3]

[1]Auburn University College of Veterinary Medicine, Merial Veterinary Scholars Research Program
[2]Auburn University Undergraduate Honors College
[3]Auburn University College of Veterinary Medicine, Department of Pathobiology

Introduction. Glioblastomas are highly lethal primary intracranial tumors that cause significant morbidity in canine and human patients. Although current treatments are ineffective in producing long-term survival benefits, temozolomide chemotherapy has been shown to prolong survival in a subset of human patients. Animal treatment has since been modeled off of human data and dosages. The present study evaluates *in vitro* chemosensitivity of three canine glioblastoma cell lines to temozolomide.

Methods. Three established canine glioblastoma cell lines, (J3T, G06A, and SDT-3G), were exposed to concentrations of temozolomide (TMZ) ranging from 0.156mM to 2.5mM. After 72 hours, cell viability was assessed using CyQUANT assays and normalized to DMSO concentration-controls. The half-maximal inhibitory concentration (IC50) of TMZ was calculated using a four-parameter variable slope curve fit nonlinear regression analysis.

Results. The IC50 (mean +/- SEM) for the cell lines was: (J3T) 0.747-mM +/-0.06, (G06A) 0.492-mM +/-0.14, and (SDT-3G) 0.416-mM +/- 0.04.

Conclusions. The *in vitro* temozolomide IC50s for these three canine glioblastoma cells were higher than those reported in temozolomide-sensitive human glioblastoma cells. These results provide some objective data for clinicians considering the use of temozolomide in canine glioblastoma patients.

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Age-Dependent Memory Response against Live Attenuated Infectious Bronchitis Virus Vaccine in Chickens

Niloofar Yaghoubi-Yeganeh, Steve L. Gulley[1], Frederik W. van Ginkel[1]
[1]Department of Pathobiology

Introduction. Infectious bronchitis virus (IBV) is endemic and currently one of the most important causes of economic losses for the poultry industry. Persistence of IBV ARK serotypes in vaccinated flocks in the southeastern US has raised questions regarding the ability of the ArkDPI-derived IBV vaccines to generate protective immunity. One possibility for the persistence of IBV is the continual mutation of the virus. Another possibility is an inadequate immune response due to early vaccination against IBV. A considerable number of chicks are currently vaccinated against IBV within the first week of life, when the immune system is largely underdeveloped leading to a limited immune response to IBV even after subsequent vaccinations. Previous research in our lab has shown that early vaccination against IBV leads to decreased levels and slower kinetics of IgA and IgG antibodies in the plasma and tears as well as decreased avidity indices for IgG antibodies. This supports our hypothesis that vaccination against IBV at an early age impairs the induction of IBV-specific immune responses leading to poor protective immunity upon subsequent IBV challenge. In this study we analyzed the vaccine induced memory response.
Methods. Birds were divided into 4 groups: IBV vaccinated at 1 day of age, age matched unvaccinated control chickens, IBV vaccinated at 3-4 weeks of age, and age matched unvaccinated control chickens. At day 28 post vaccination birds were euthanized and spleen and head-associated lymphoid tissue (HALT), i.e., Harderian gland and CALT (conjunctiva-associated lymphoid tissue), were collected. Tissues were homogenized through a cell dissociation sieve and underlayed with histopaque to isolate lymphocytes. Lymphocytes were pelleted then re-suspended in Ariaans RPMI and then manually counted using a hemocytometer. Splenic lymphocytes and HALT lymphocytes were cultured and stimulated with LPS (lipopolysaccharide) for 6 days. Supernatants were collected at day 6 and immunoglobulin levels were measured via an IBV-specific ELISA. Additionally, splenic B lymphocytes were isolated and stained with Bu-1-FITC and biotinylated IBV followed by streptavidin-Alexa660 and analyzed by flow cytometry to determine the relative abundance of IBV-specific memory B lymphocytes in the lymphoid tissues of these age groups.
Results. ELISA results of culture supernatant of LPS stimulated lymphocytes showed an increase in IgA and IgG secretion by memory or effector B cells from HALT vaccinated chickens compared to control chickens, but failed to show a significant difference between younger and older vaccinated groups. The spleen lymphocytes did not secrete IBV-specific antibodies after LPS stimulation regardless of age. However, flow cytometric analyses of IBV-specific B cells in the spleen showed a significant increase in older IBV vaccinated birds compared to control chickens and day 1 vaccinated chickens.
Conclusions. Based on our analyses the LPS induced IBV-specific antibody recall responses do not significantly differ between birds vaccinated at day 1 of age versus 3-4 weeks of age in HALT while no recall response was observed in splenic tissue. This indicated that the B cell memory response is predominantly located in HALT rather than spleen. In contradiction with this observation is the flow cytometry data that showed significantly higher IBV-specific B cells in the spleen of older birds but not day 1 vaccinated bird. The latter observation confirms our hypothesis while the former does not. It is possibly that 4 weeks after vaccination HALT still contains considerable numbers of plasma cells secreting IBV-specific antibodies rather than activating memory B cells. An issue we will address in future experiments.
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Graduate Student Poster Presentations

Concurrent Cutaneous Phaeohyphomycosis And Nocardiosis In A Dog

J. Bailey[1], J. Koehler[1], A. White[2], C. Lawson[1]

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

Introduction. A rare presentation of concurrent phaeohyphomycosis and nocardiosis in a dog is discussed highlighting the importance of culture and histochemical stains for the identification and definitive diagnosis of these highly pathogenic organisms.

Abstract. A 6-year-old spayed female Doberman Pinscher with a previous diagnosis of pemphigus foliaceus presented with a two-week history of multifocal exudative and hemorrhagic skin nodules, cellulitis of the face and left hind carpus, and pitting edema of the left hind limb and right forelimbs. Microscopically, pigmented fungal hyphae were present within the lumen of hair follicles and the keratinized layer of the epidermis, as well as free within the dermis and subcutis. Also present within the dermis were scattered colonies of weakly Gram-positive, variably beaded, long filamentous rods. Both organisms were associated with multinodular pyogranulomatous inflammation. *Curvularia* sp., *Nocardia* sp., *Streptococcus canis*, and *Staphylococcus intermedius* were all isolated from the cutaneous lesions. This patient's history of high doses of immunosuppressive drugs for treatment of pemphigus foliaceus likely played a role in development of this unusual multiorganism opportunistic infection.

Discussion. Both cutaneous phaeohyphomycosis and nocardiosis, while well documented in veterinary medicine, are considered rare diseases in the dog. The etiological agents responsible for these diseases are considered opportunistic and infection in healthy animals is uncommon. Even more rare are cases of concurrent nocardiosis and phaeohyphomycosis. There is one documented case in a person[1]. In most cases of phaeohyphomycosis and nocardiosis, the organisms are thought to be inoculated into sites of infection by trauma. However, there is a documented case of phaeohyphomycosis in an immunocompromised dog without evidence of trauma, and fungal organisms were commonly present in the lumen of hair follicles[2]. Our case mirrors this as our patient was immunocompromised, had no history of trauma, and fungal organisms were found in the hair shaft of many follicles. This suggests that in the current case the follicular epithelium was a likely route of entry for these organisms. Another group of fungal organism known to colonize hair follicles and cause extensive granulomatous inflammation in immunocompromised animals are dermatophytes. While these organisms usually are considered non-pigmented, dermatophytes have been documented to produce melanin or melanin-like compounds in vitro and during infection[3,4]. Because of this, a definitive diagnosis of phaeohyphomycosis should be made based on both histology and fungal culture results.

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Characterization and microRNA profiling of canine mammary tumor exosomes

Eric J. Fish[1], Patricia DeInnocentes[1], Farruk L. F. Kabir[1], and R. Curtis Bird[1]

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

Introduction. Most deaths from breast cancer in women and dogs with canine mammary tumors (CMT) occur as a result of metastatic disease, so early detection ahead of this event is the key to reducing morbidity and mortality. Multiple types of tumors have been shown to elaborate extracellular vesicles (exosomes) that contain microRNA (miRNA, miRs). miRs are stable in biofluids and differentially expressed in cancer. Previous tissue microarray data of CMT cell lines from our lab indicates several miRs, including miR-141 and miR-21, are significantly upregulated in CMT relative to normal mammary epithelial cells.

Methods. Multiple CMT cell lines (CMT-12, 27, 28, 47, 119) and normal mammary cells (HMEC and CMEC) were grown in Xerum-free™ medium plus DMEM. Media was harvested and progressively processed to remove cells, organelles, bacteria, and debris. This cell-free fraction was evaluated by transmission electron microscopy (TEM) and dynamic light scattering (DLS). RNA was extracted and cDNA specific for miR-16, -21, and -141 was created. RT-qPCR for these miRs was performed.

Results. TEM showed the cell-free fraction contained round membrane-bound particles ranging from 10-200 nm. DLS showed the majority of these particles were ~62 nm in diameter. miR-21 was amplified from exosomal RNA from CMT-12, 27 and 47, but not CMT-28 cells or exosomal RNA. miR-16 was amplified from exosomal RNA from all cell lines relatively consistently (average Ct ~25). miR-141 was amplified at about 1.0-fold relative expression to miR-16 in CMT-12 and CMT-27, was barely detected in miR-119, and was not found in any other cells.

Conclusions. These results indicate that cell-free fluid from CMT cell culture contains extracellular vesicles, and miRs, including miR-16, 21, and 141, the can be successfully amplified from this fluid. Importantly, miR-141 was only detected in the neoplastic lines, but not normal controls. Future work is necessary to define the expression, pathologic role, and specificity as a biomarker, of miR-141 in canine cancers.

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Creating a Reproducible and Quantifiable Behavioral Model of Mitochondrial Dysfunction in the Rat

Michelle E. Hoffman, Shannon N. Helsper, and Michael H. Irwin*

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
*Corresponding author

Introduction. The pathological effects of mitochondrial dysfunction result from both oxidative damage and bioenergetic deficiency, and are more severe in cells and tissues with high metabolic energy demands such as neurons, skeletal muscle and cardiac muscle. In this context, our research efforts have focused on a group of proprietary synthetic lipoylcarnitine and butyrylcarnitine derivatives (PMX compounds) as potential therapies to minimize oxidative damage and maximize mitochondrial energy production in animal and cellular models of mitochondrial disease. An additional animal model was required to further evaluate these compounds, and the rotenone-induced rat model of Parkinson's disease was selected for its established inhibition/disruption of mitochondrial complex I. Adapting this model to achieve a measurable behavioral phenotype that does put the rat into an overly depressed state has been challenging, requiring continual monitoring, evaluation, and adjustment to the model.

Methods. Lewis rats are acclimated and trained for manual restraint prior to the experiment. They are then dosed daily via intraperitoneal injection with rotenone at varying concentrations for a period of two-three weeks to induce a Parkinson's disease (PD)-like state. At completion of dosage regimen, rats are evaluated through neuromotor testing to assess their coordination and stamina. Following data collection necropsy is performed and tissues harvested for immunohistochemistry and protein expression assays. Early cohorts were composed of only control (untreated) and rotenone-treated groups to validate the model. Subsequent cohorts of rotenone-treated rats were co-treated with PMX compounds, which were designed as potential therapeutic compounds for mitochondrial dysfunction.

Results. Experiments are ongoing, but preliminary results are promising. Initial cohorts showed a significant decrease in speed and distance traveled by rotenone- treated rats in the Open Field Test, but rats co-treated with PMX compounds did not show any significant improvement in neuromotor tasks. However, post-mortem immunohistochemistry of brain tissue (tyrosine hydroxylase immunolabeling of substantia nigra *pars compacta* - the area of the brain affected in PD) demonstrated neuroprotection for rats co-treated with PMX compared to rotenone-only treated animals.

Conclusions. Data collected from early cohorts suggest that despite creating a physiological change in the brain, we have not yet captured an observable neuromotor or behavioral phenotype. Further testing is underway, and will provide important insight into relevant mechanisms in this rodent model of mitochondrial dysfunction-induced PD/neurodegeneration.

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***In vitro* anti-tubulin effects of benzimidazole anthelmintics mebendazole and fenbendazole on canine glioblastoma cells**

Serene R. Lai[1] and Jennifer W. Koehler[2]

Auburn University College of Veterinary Medicine. Departments of [1]Clinical Sciences and [2]Pathobiology

Introduction. Benzimidazole anthelmintic drugs have been reported to have antiproliferative effects on several cancers both *in vitro* and *in vivo*, with reduced off-target toxicity as compared to other microtubule-disrupting drugs. The purpose of this study was to evaluate the *in vitro* chemosensitivity of canine glioblastoma (J3T) cells to mebendazole (MBZ) and fenbendazole (FBZ).

Methods. Cells were exposed to drugs for 72 hours, and then cell viability was evaluated using the MTT assay. The half-maximal inhibitory concentration (IC_{50}) of MBZ and FBZ was calculated using a four-parameter variable slope curve fit nonlinear regression analysis. Western blot was used to compare the ratio of polymerized to depolymerized tubulin between drug-treated cells and untreated controls, and between the two drugs.

Results. The mean IC_{50} of MBZ and FBZ were 0.026 (range of 0.024 - 0.03) and 0.55 μM (range of 0.53 – 0.57) respectively. Treatment with MBZ and FBZ resulted in increased depolymerization of tubulin than in the untreated control.

Conclusions. Our *in vitro* data suggest that MBZ and FBZ may be good candidates for treatment of canine glioblastomas. Further *in vivo* studies are required.

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Expression of Mammaglobin-A in canine tumors.

Gisela Martinez-Romero[1], Patricia DeInnocentes[1], R. Curtis Bird[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn, AL

Introduction. Canine mammary gland tumor and human mammary tumors have many molecular and biological similarities. The evolution of breast cancer is accompanied by multiple changes in gene expression. Identification of these genes and elucidation of the mechanism controlling their patterns of expression could lead to novel diagnostic and therapeutic targets for the clinical management of breast cancer. An example of these genes is the mammaglobin gene (MGBA). MGBA encodes mammaglobin-A, whose mRNA is upregulated in primary breast tumors. Mammaglobin-A has unique properties that make it an exceptional target for cancer vaccine therapy: 1) it is expressed almost exclusively in breast cancer, 2) it is overexpressed in 40-80% of primary breast cancers, 3) and overexpression is evident in noninvasive, invasive, and metastatic breast cancer. We have explored the expression profile of MGBA in canine breast cancers.

Methods. Cell culture: Six established canine mammary tumors cell lines (CMT 9, 12, 27, 28, 47, 119) and one canine mammary epithelial cell line (CMEC) were grown in Alpha-MEM supplemented with 10% FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin.
RNA extraction: Total cellular RNA was extracted from the cell lines using the High Pure RNA isolation kit according to the manufacturer's instructions.
RT-PCR analysis of total RNA: RNA was next analyzed by reverse transcriptase-PCR (RT-PCR) using the Access RT-PCR system according to the manufacturer' s instructions. The forward primer for mammaglobin-A was 5'-ATGAAGCTGCTGAGAGTCCTTGTGCTG-3' and the reverse primer was 5'- TGCTGAGAGTCCTTGTGCTGGTTGCC-3. Reverse transcription was performed for 45 minutes at 48° C. The cDNA amplification program consisted of an initial denaturation step (94° C for 2 minutes) followed by 40 amplification cycles of denaturation (94°C for 30 seconds), annealing (60°C for 1 minute) and extension (68°C for 1 minute). A final extension at 68°C for 7 min was included. The DNA was purified using the QiaQuick PCR purification kit (Qiagen). Equivalent volumes of each purified PCR product DNA were resolved on a 2.5% agarose ethidium bromide-stained gel. In order to confirm the nucleotide sequence of the amplified products (CMT cell lines), purified PCR product DNA was sequenced (Dana-Farber Cancer Center DNA Resource Core) and compared to Genbank mammalian MGBA sequences.

Results. In all CMT cell lines and the CMEC cell line, the mammaglobin PCR DNA product was detected on ethidium bromide-stained agarose gels as the expected 237 bp fragment. The specificity of the amplified product was confirmed in all the CMT cell lines, except the CMT 47 cell line, by DNA sequencing. The sequence obtained for the amplified product was identical to the known canine mammaglobin cDNA sequence.

Conclusions. Mammaglobin-A was expressed in all 6 established CMT cell lines and in CMEC cells. To our knowledge, there are no studies that have analyzed the expression of Mammaglobin-A in CMT cell lines. This is a first approach to analyze the relevance of Mammaglobin-A in canine mammary gland cancer and to assess its value as a promising therapeutic agent in dogs. Mammaglobin-A is a secreted protein that has been detected in the sera of breast cancer patients. These properties make mammaglobin an ideal candidate as a clinical marker for breast cancer detection.

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Sustained-Release Voriconazole Hydrogel for Ocular Use in Horses: Safety and *in vivo* studies.

Mariano Mora[1], Eva M. Abarca[1], Sue H. Duran[1], Anne A. Wooldridge[1], Jey Koehler[2], Stephanie Shrader[2], and Jayachandra B. Ramapuram[3]

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

Introduction: Corneal fungal infection (keratomycosis) is a sight-threatening disease in horses. Treatment of keratomycosis is challenging, expensive, lengthy and intensive. Subconjunctival (SC) administration of a slow-release, thermosensitive, biodegradable voriconazole thermogel may allow for sustained delivery of therapeutic concentrations of voriconazole to the cornea; therefore increasing efficacy and decreasing frequency and cost of treatment of equine keratomycosis. There is a need to assess the safety of different concentrations of the voriconazole thermogel, at different time points in the equine cornea.

Methods: An *ex vivo* trans-corneal permeation model was used to assess the effects of the voriconazole thermogel at two different concentrations, 1 mg and 5 mg. Fresh equine cornea sections were exposed to 1 mg and 5 mg voriconazole thermogels and compared to plain hydrogel (control) and physiological buffered saline (PBS, control) at different time points. Histological analysis of the samples was performed after 2, 4, 6, and 8 hours of exposure. Morphometric analysis was performed on all the cornea sections, and the different groups were compared for structural changes.

Results: Measurements performed to the tissue samples was inaccurate due to the presence of artifacts. Using this scientific method to assess the effects of the voriconazole hydrogel to the equine corneas yielded inconclusive results.

Conclusions: The trans-corneal permeation model is inappropriate for testing corneal toxicity. The presence of artefacts limited an accurate morphometric analysis of the tissues at all time points.

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Evaluation of commercial glucometer test strip for measurement of glucose in tears and potential correlation with blood glucose in dogs

Roxanne Rodriguez Galarza, Eva Abarca, Rafael Obrador and Lenore Bacek

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

Introduction.
Diabetes mellitus is one of the most common endocrine diseases diagnosed in dogs. Long term monitoring is necessary to ensure adequate blood glucose concentrations. In people as well as in dogs, this can be done by obtaining a drop of blood and using a portable glucose monitor. The discomfort that results in repeated blood draws can limit compliance and lead to suboptimal blood glucose control. A pain-free, noninvasive technique such as monitoring tear glucose levels would improve compliance and outcome.

Methods.
Client owned dogs that presented to Bailey Small Animal Teaching Hospital consented to tear collection and ophthalmologic exam. Dogs included had normal ocular surface. Tears were collected from the lacrimal lake using microcapillary tube. The sample obtained was used to measure tear glucose using a portable glucose monitor (AlphaTRAK 2 Abbot Diabetes Care Inc, Alameda CA,US). Tear glucose was compared to glucose blood level (from standard bloodwork, glucose curve, etc.).

Results.
A total of six glucose curves from five diabetic dogs were obtained with a total of 28 readings. A total of four single reading were obtained from four non-diabetic dogs. In diabetic dogs, mean blood glucose was 347.7 mg/dL (Range 60-687 mg/dL:, Median 323 mg/dL) and mean tear glucose was 25mg/dL (Range: <20- 79 mg/dL; Median 28 mg/dL). In non-diabetic dogs, mean blood glucose was 117.25 mg/dL (Range: 107-126 mg/dL; Median 118 mg/dL) and tear glucose was not measurable with AlphaTRAK glucometer (<20 mg/dL).

Conclusions. Results suggest that glucose can be measurable in the tears if elevated >300 mg/dL in blood. Measuring glucose in the tears can be an easy, less invasive way to determine hyperglycemic state. Additional data will need to be recorded to determine if there are differences detecting glucose in tears among diabetic individuals as well as patients with ocular surface disease.

Genetic Modification To Achieve Targeting Of Adenoviral Vectors To Malignant Cells Of Lymphocyte Origin

Abdul Mohin Sajib[1], Dr. Payal Agarwal[1,2], Dr. Maninder Sandey[1,2], Dr. Bruce F. Smith[1,2]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn
[2]Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, Auburn, AL, US

Corresponding author: Dr. Bruce F. Smith (smithbf@auburn.edu)

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

Growth and Function of Equine Endothelial Progenitor Cells Labeled with Semiconductor Quantum Dots

Randolph L. Winter[1], Wen J. Seeto[2], Yuan Tian[2], Fred J. Caldwell[1], Elizabeth A. Lipke[2], and Anne A. Wooldridge[1]

[1]Department of Clinical Sciences, Auburn University, AL
[2]Department of Chemical Engineering, Auburn University, AL

Introduction. Endothelial progenitor cells (EPCs) contribute to neovascularization and vascular repair in vivo, and therefore are attractive for clinical use in ischemic disease. Tracking of stem and progenitor cells is essential to determine engraftment after administration. Semiconductor quantum dots (QD) are promising for cell labeling due to their ease of uptake by many cell lines and their continued presence after many cell generations. The purpose of this study was to evaluate function and growth of equine EPCs after QD label.

Methods. Equine EPCs were incubated with QD (2 – 20nM) for 12-hr or 24-hr, and intensity of label was assessed with fluorescent microscopy. Cell proliferation of EPCs labeled with QD for optimum time and concentration was then assessed by comparing the number of cell doublings per day (NCD) and the population doubling time (PDT) in labeled and unlabeled cells. Function of EPCs was assessed by comparing uptake of acetylated low-density lipoprotein (DiO-Ac-LDL) and tubule formation on growth factor containing matrix in labeled and unlabeled cells.

Results. Equine EPCs readily labeled with QD, showing maximum fluorescence using 20nM QD, with a 24 hr label-contact time. NCD for unlabeled EPCs was 1.90 and 1.89 for labeled cells ($p=0.95$). PDT was 29.32 hrs for unlabeled cells and 29.34 hrs for labeled cells ($p=0.99$). Uptake of DiO-Ac-LDL by EPCs was not affected by the presence of QD label (97.9% uptake for QD labeled cells; 97.0% uptake by unlabeled cells). Tubule formation on Matrigel was not affected by the presence of QD label.

Conclusions. Equine EPCs are effectively labeled with QD, and QD concentrations up to 20nM do not affect cell growth or function. The use of QD labeling with equine EPCs may be an ideal way to track engraftment of EPCs in clinical applications.

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Post-graduate/Faculty Poster Presentations

Evaluation of a conditionally replicative adenoviral vector for the treatment of canine osteosarcoma

Payal Agarwal and Bruce F. Smith

Scott Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL

Introduction: Appendicular osteosarcoma (OSA) is an aggressive bone cancer, accounting for around 80% of all canine bone tumors. Even with the standard-of-care therapy of amputation and chemotherapy, the prognosis is poor, with most dogs dying due to tumor spread (metastasis) within one year, and less than 20% surviving to 2 years following diagnosis. We have developed a conditionally replicating adenoviral vector (CRAD) that can be both safely administered to patient dogs and have potential efficacy in treating osteosarcoma. We hypothesize that administered CRAD will both kill tumor in the short-term via cytopathic effect and incite an antitumor immune response when cryptic tumor associated antigens are recognized in the context of an anti-vector immune response that may in turn protect the patient from relapse long-term, and so improve disease-free intervals and survival times in response to virotherapy. We have examined the interaction of this virus with the immune system of dogs, including assessing any potential increase in immune response to the tumor.

Methods: 10 Patient dogs with a confirmed diagnosis of osteosarcoma were enrolled in the clinical trial and were treated with the virus following limb amputation. Blood, free catch urine and feces were collected from patient every 12 hrs. for six days post vaccination for screening of virus loading and shedding. Primary osteosarcoma cells, collected from the tumor mass post limb amputation, were cultured. Peripheral blood mononuclear cells from patient blood samples were collected pre and post treatment and were analyzed for dendritic and regulatory T-cell numbers. Humoral and cellular responses to tumor and virus were also evaluated pre and post treatment using blood collected from enrolled patients.

Results: Ten dogs were enrolled in the current study. Two dogs were long-term survivors, living for more than a year. No adverse events were observed in the trial. No dogs showed viral genome shedding in urine or feces. Humoral immune responses against OSA were observed pretreatment in most dogs and showed alterations post-treatment in some dogs as analyzed by western blot and flow cytometry.

Conclusions: 20% of dogs in this study were long survivors. The lack of virus shedding in blood, urine and feces shows that the virus is safe in patients and is only conditionally replicative in osteosarcoma cells. The virus appears to alter the humoral response against osteosarcoma cells in at least 4 dogs enrolled in the clinical trial, which indicates a potential immune response against tumor antigens.

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A Novel Inherited Cerebellar Abiotrophy in a Cohort of Related Goats

J.W. Koehler[1], B.W. Newcomer[1,2], M. Holland[2], J.M. Caldwell[2]

1. Department of Pathobiology, Auburn University
2. Department of Clinical Sciences, Auburn University

Introduction. Cerebellar abiotrophies, also known as cerebellar ataxias, are characterized by premature postnatal degeneration of cerebellar neurons. Presented here are the clinical, magnetic resonance imaging (MRI), gross, and histopathological features of a novel inherited cerebellar abiotrophy in a cohort of five closely-related mixed-breed goats (*Capra hircus*) in the southeastern United States. Three animals were evaluated here, while two remain alive but symptomatic on the farm. The animals all presented with early juvenile-onset ataxia, hypermetria, wide-based stance, head tremors, and nystagmus.

Methods. MRI was used to evaluate the brain *in vivo*. Serum chemistry profiles, complete blood counts, cerebrospinal fluid analysis, serum copper levels, and caprine arthritis and encephalitis virus titers were analyzed and compared to published reference intervals. Necropsy evaluation of the gross and microscopic pathology was performed to characterize the extent, distribution, and nature of the cerebellar lesions.

Results. On MRI and at gross examination, there was moderate thinning of the cerebellar vermis and sharpening of the folia. Histologically, the vermis, paravermis, and flocculonodular lobe had moderate to severe segmental loss of Purkinje cells with sparing of the hemispheres and secondary loss of granule cells and astrogliosis.

Conclusions. The clinical presentation, pattern of inheritance, MRI findings, and gross and histopathological findings in these cases support the diagnosis of inherited cerebellar abiotrophy in this cohort of closely-related goats. Gross and histopathological changes, including the appearance and distribution of lesions within the cerebellum, are similar to what has been described in humans, other domestic animal species, and several genetically-engineered or spontaneous rodent models of cerebellar ataxia. Heritable cerebellar ataxias have been reported in many domestic animal species but have never previously been reported in goats. Genomic DNA from multiple affected and unaffected goats in the herd has been collected and is awaiting further investigation to characterize the defect at the genetic level.

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Evaluation of Delayed Insemination with Sexed Semen for Non-responding Beef Heifers after Estrus Synchronization

Benjamin W. Newcomer[1], Chance L. Armstrong[2], Kay P. Riddell[1], Misty A. Edmondson[3], and Paul H. Walz[1]

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

Introduction. The objective of this study was to compare estrus synchronization and fixed-timed artificial insemination (FTAI) protocols in commercial beef heifers to be bred with sexed semen. Secondly, the trial evaluated the benefit of delaying insemination for 24 hours in heifers not demonstrating estrous activity following synchronization and prior to FTAI.

Methods. A group of 120 Angus-cross heifers were stratified by reproductive tract score and randomly assigned to one of two synchronization protocols: 1) 5-day Co-Synch + CIDR or, 2) 7-day Co-Synch + CIDR. Estrous activity was monitored using a wireless heat detection system. Insemination in both groups was delayed 24 hours in heifers that did not demonstrate estrus in response to synchronization. Heifers demonstrating estrous activity in the 28 days following FTAI were re-inseminated using sexed semen.

Results. The numbers of heifers demonstrating estrus in each group were similar ($p = 0.47$). In group 1, 10/60 heifers were pregnant to FTAI; 18/60 were pregnant to FTAI in group 2 ($p = 0.132$). Conception rates for heifers demonstrating, or not demonstrating estrous activity were 29.2% and 16.4%, respectively ($p = 0.151$). More heifers in group 1 demonstrated estrus and were pregnant to sexed semen in the 28-day period following FTAI resulting in an equal number of pregnancies in both groups at the end of the study.

Conclusions. This study further confirmed suboptimal pregnancy rates when using sexed semen compared to conventional semen in FTAI protocols, even with estrus detection. Further study is warranted to explore the observed differences in conception rates.

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