

## Student Abstracts 2008



### Trainees

Front row (L to R): Jennifer Sims, Jenna Loar, Kristin Farmer, Leslie Koenig

Second row (L to R): Talisha Moore, Jamesia Showers, Courtney Bruner

Third row (L to R): Kimberly Lednum, **Chasie Johnson**, Virginia Munson, Mary O. Aboko-Cole, Karen Barger, and Nadine Kirk

Fourth row (L to R): Claire Fellman, Alexis Bardzinski, Kasey Hall, Shayla J. Belton, Brook Bobo, and Jason Gray

Faculty mentors

Fifth row (L to R): Dr. Andrew Mackin, Dr. Erdogan Memili, Dr. Jan Chambers, Dr. Peter Ryan, and Dr. Scott Willard

Sixth row (L to R): Dr. Bob Wills, Dr. Robert Meyer, Dr. Mark Lawrence, Dr. Andrea Varela-Stokes, Dr. Karen Coats, and Dr. Jeff Eells

### **Closure of gaps in the *Flavobacterium columnare* genome**

Mary O. Aboko-Cole<sup>1</sup>, Mark L. Lawrence<sup>2</sup>, Attila Karsi<sup>2</sup> <sup>1</sup>School of Veterinary Medicine, Tuskegee University, Tuskegee, AL <sup>2</sup>Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Columnaris disease caused by the *Flavobacterium columnare* is the second leading cause of mortality in the channel catfish industry, resulting in major economic losses. At present, no effective treatment for columnaris is available. Previous work in our ongoing research produced a genome sequence of this pathogen with less than twenty gaps. Accordingly, the focus of this study is to close those existing gaps. To achieve this, the first step was to design primers from the ends of the contigs and amplify the gaps by polymerase chain reaction (PCR). The products created were used as templates in subsequent sequencing reactions. New sequences were obtained by primer walking and assembled to existing genome to close the gaps. With this strategy, we were able to successfully close all but one gap with the paired end information. Remaining contigs without scaffold information are expected to be joined using multiplex PCR followed by primer walking. A complete genome sequence without any gaps should enable functional genomics research and accelerate hypothesis-driven research on this pathogen that affects multiple fish species.

Student Support: NIH grant #5T35RR007071 Research Support: USDA CSREES grant #2006-35600-16571

### **Proteomic Evaluation of Equine Recurrent Airway Obstruction as an Asthma Model**

Bardzinski, Alexis; Costa, Lais R. R.; Fiona, McCarthy; Nanduri, Bindu; Burgess, Shane; Swiderski, Cyprianna College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Equine recurrent airway obstruction (RAO), a naturally occurring obstructive pulmonary disease, occurs as a winter-associated disease in stalled horses exposed to hay, and a summer form (SPARAO) affecting horses on pasture in the southeastern US. RAO shares strong clinical similarity to human asthma. However, in RAO the bronchoalveolar lavage fluid (BALF) indicates a neutrophilic inflammation (NI), while human asthma has a predominately eosinophilic response with a subset of affected patients exhibiting NI. Mounting evidence indicates an inflammatory pathway including TLR4, TH17, IL-17 and IL-8 mediates NI in human asthma. We hypothesize this same pathway mediates equine RAO, accounting for the NI in RAO. Utilizing the recently sequenced equine genome, we have combined hypothesis generating and hypothesis driven approaches to study NI in SPARAO. Using the equine genome sequence we have completed a proteomic survey of affected versus non-affected equine BALF. Data analysis allows us to detect key responses in SPARAO horses and generate hypotheses about this condition. Using a hypothesis-driven approach we have examined RAO literature to find evidence for the TLR4/TH17 pathway in RAO, and sought to determine if the pathway is supported by proteomic analysis of BALF samples. We elucidated equine orthologs for human/mouse genes in the pathway and used functional analysis and published literature to provide Gene Ontology (GO) for these products. Functional annotation using GO facilitates physiological modeling of complex biological questions. Our combined approach allows identification of novel processes critical for SPARAO and confirmation of pathways known to be significant in clinically similar disease processes.

Student Support: NIH grant #5T35RR007071

### **Annotation of *Escherichia coli* Plasmid with an Analysis of Virulence-Conferring Genes**

Shayla J. Belton<sup>1</sup>, Michele L. Williams<sup>2</sup>, Tim Johnson<sup>3</sup>, Lisa K. Nolan<sup>4</sup>, and Mark L. Lawrence<sup>2</sup> <sup>1</sup>School of Veterinary Medicine, Tuskegee University, Tuskegee, AL <sup>2</sup>Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS <sup>3</sup>Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN <sup>4</sup>College of Veterinary Medicine, Iowa State University, Ames, IA

Colisepticemia is an intestinal disease of neonatal calves caused by certain serotypes of *Escherichia coli* possessing virulence factors that enable them to cross mucosal surfaces and produce pathology. The virulence plasmid, pVir, was identified in an *E.coli* strain that was isolated from a 2006 case of bovine septicemia. In this collaborative project between Iowa State University and Mississippi State University, the 138 -kb plasmid was sequenced and submitted for automated annotation by the J. Craig Venter Institute's Annotation Service. The plasmid sequence was then manually annotated using MANATEE (JCVI). Assigned annotations for all 203 identified open reading frames were analyzed using sequence evidence and literature searches, and a function, process and component were assigned to each gene product. Particular attention was paid to known genes conferring virulence to *E.coli*, such as cytotoxic necrotizing factor 2 (cnf2) and cytolethal distending toxin type III (cdtIII), whose products alter mammalian cell signal transduction and cause target cell cycle arrest, respectively. In addition, pVir was found to contain an F-17 fimbrial operon, a second, potentially novel, fimbrial operon, as well as two hemolysin genes. The occurrence of hemolysin genes along with cnf2 on plasmid Vir is a point of particular significance. Previous literature provides supporting evidence that most cnf2-producing strains are not hemolytic. The cnf1 gene is primarily associated with strains possessing the hemolysin operon. Annotation of pVir led to increased understanding regarding disease-causing mechanisms of colibacillosis in calves.

Student and Research Support: NSF grant #0626667

### **Effects of Genistein on Gene Expression & Epithelial Growth in the Neonatal Porcine Cervix**

B. A. Bobo<sup>1</sup>, J.N. Feugang<sup>2</sup>, E.L. Schenck<sup>2</sup>, N. M. Kirk<sup>1</sup>, P.L. Ryan<sup>1,2</sup> <sup>1</sup>College of Veterinary Medicine and <sup>2</sup>Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS 39762

Phytoestrogens (i.e., genistein) are non-steroidal plant compounds that are found in very high concentrations in soy-based products, such as infant formulas, and can mimic endogenous estrogens. Questions have arisen regarding the potential adverse effects of increased dietary phytoestrogen intake on fetal and neonatal reproductive development. The objective of this study was to examine the effects of genistein on relaxin receptors 1 and 2 (RXFP 1, 2) gene expression and epithelial growth in the neonatal porcine cervix. Gestating sows (d 100) were supplemented (1.5 mg/kg BW/d) with (n = 8) or without (n = 8) a dietary soy extract (Novasoy70%) for the remainder of the pregnancy and lactation. On post natal day 7 (PND 7), neonatal pigs were assigned to one of four oral treatments; control (CNT, vehicle), low genistein (LG, 3 mg/kg BW), high genistein (HG, 9 mg/kg BW) or estradiol 17 $\beta$  (E2; 50  $\mu$ g/kg BW) from PND 7 to 14. On PND 14, 4 piglets/treatment were euthanized and cervixes collected for histological and gene expression analyses. Tissue was stained (H&E) to measure luminal epithelial height or RXFP 1 and 2 gene expression quantified by RT-PCR. GLM procedures of SAS were used to analyze data. There was an increase in cervical luminal epithelial height in the Novasoy sow-LG piglet group (p<0.05) when compared to all others except the Novasoy sow-HG group. There were no observable changes with respect to RXFP 1 and 2 expression. While changes in RXFP receptor expression was not observed, increase in epithelial height indicate a need to examine other genes (i.e., Hoxa 10, Wnt4) and the effect that phytoestrogens may have on their regulation.

Student Support: NIH Grant #5T35RR007071 Research Support: USDA-ARS Biophotonics Initiative #58-6402-3-0120

### **Flow Cytometric Determination of Optimal Agonists for Various Canine Platelet Markers**

Bruner, Courtney; Thomason, John; Pinchuk, Lesya; Pruett, Stephen; Stokes, John; Lunsford, Kari; Mackin, Andrew College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Platelet activation is crucial for normal canine hemostasis. Several pathological conditions and even drugs can result in a hypercoagulable state and a potentially life-threatening thromboembolic complication. The detection of surface markers on activated platelets may provide valuable information in determining the cause of thromboembolic diseases. The aim of this study was to determine both the optimal platelet activator and concentration for detection of various platelet markers. To induce a hypercoagulable state *in vitro*, platelet agonists were used prior to evaluation with flow cytometry. EDTA or citrated whole blood was incubated with varying concentrations of several agonists including IBOP, epinephrine, platelet activating factor (PAF), phorbol 12-myristate 13-acetate (PMA), and adenosine 5'-( $\alpha$ ,  $\beta$ -methylene) diphosphate (ADP). We investigated several platelet markers by comparing either the mean fluorescent intensity of samples or the percent of cells gated prior to and after activation with the various agonists. I-BOP and a combination of epinephrine and I-BOP upregulated expression of CD9 by 20% and 34% respectively, while expression was downregulated by 26% using platelet activating factor. The agonist PMA increased the expression of P-selectin by 82%, while the agonist PAF only increased P-selection expression by 9%. Identification of drugs that may cause hypercoagulable states may help direct medical intervention in predisposed states.

Student Support: NIH Grant # 5T35RR007071 Research Support: Dr. Hugh Ward Discretionary Fund

### **Epigenetic control of CD30 expression in a unique natural animal model of CD30 over-expressing lymphomas**

Kristin Farmer<sup>1</sup>, Dusan Kunec,<sup>1,2</sup> and Shane C. Burgess<sup>1,2,3,4</sup> <sup>1</sup>College of Veterinary Medicine, <sup>2</sup>Mississippi State University Institute for Digital Biology, <sup>3</sup>Mississippi Agriculture and Forestry Experiment Station, <sup>4</sup>MSU Life Sciences and Biotechnology Institute, Mississippi State University, Mississippi State, MS

Marek's disease virus (MDV) is a highly contagious, cell-associated alpha-herpesvirus of chickens that causes widespread lymphoma development (Marek's Disease [MD]). Although all chicken genotypes are susceptible to MDV infection and cellular neoplastic transformation, only susceptible genotypes develop clinical lymphomas. MD is not only one of the most economically important diseases of poultry but it is also a unique natural biomedical model for human lymphomas that over-express the CD30 antigen. MDV encodes the Meq oncogene (a functional analogue of the Epstein-Barr Virus [EBV] oncogene LMP-1) essential for transforming T cells. Both MDV Meq and EBV LMP-1 have convergently evolved to increase CD30 expression: Meq does so by increasing transcription at the CD30 promoter. We know that Meq effects CD30 transcription differently in MD resistant and susceptible chicken genotypes. Here we test the hypothesis that this differential transcription is due to differential methylation. We are doing methylation analysis of 2.5 Kb of the CD30 promoter. Because DNA methylation usually occurs at CpG sites we are specifically examining three regions with high numbers of CpGs using pyrosequencing. Before pyrosequencing, the DNA is treated with bisulfite to convert unmethylated cytosines to uracils, leaving methylated cytosines unaffected. During pyrosequencing, DNA polymerase adds complementary dNTPs onto the template, which releases pyrophosphate (PPi).

Student Support: NIH grant #5T35RR007071

### **Identification of Cyclosporine A Targets in Activated Canine T-Cells by Flow Cytometry**

Fellman, Claire; Archer, Todd; Pinchuk, Lesya; Pruett, Stephen; Stokes, John; Lunsford, Kari; Mackin, Andrew College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Cyclosporine A is an immunosuppressive drug that inhibits the calcineurin-dependent pathway of T-cell activation. In this study, we assessed potential targets of immunosuppression in activated canine T-cell populations by flow cytometric analysis. Peripheral blood mononuclear cells were separated using density gradients and cultured in the presence of either concanavalin A or PMA and Ionomycin. The expression of the surface T-cell activation marker CD95, and intracellular cytokines, IL-2, IL-4, and IFN $\gamma$ , were analyzed in resting and activated T-cells by flow cytometry. Activated CD3<sup>+</sup> T-cells showed increased expression levels of CD95, IL-2, IL-4, and IFN-  $\gamma$  compared to control T-cell populations. These findings were further tested with *in vitro* with cyclosporine A at 100 and 200ng/mL, and there was suppression of all markers, particularly the cytokines. An *in vivo* pilot study with dogs undergoing cyclosporine A therapy showed similar results, suggesting suppressed

expression of the markers related to T-cell activation could eventually be used as an indicator of the efficacy of cyclosporine A therapy, thus identifying the best way to utilize this drug for immunosuppression in clinical patients.

Student Support: NIH Grant #5T35RR007071 Research Support: Dr. Hugh Ward Discretionary Fund

### **Distribution of *Salmonella* Serotypes through the Broiler Production Continuum**

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This study follows 76 broiler flocks from 38 farms from the date of chick placement in the grow-out house to the final stages of the processing plant in an attempt to map the flow of specific *Salmonella* serotypes along the production continuum. *Salmonella* were isolated from 24.8% (5,384/21,671) of the samples; 5,256 of the isolates were serotyped, and a total of 71 different serotypes were identified. The greatest percentage of these isolates (19%) came from the paper liners of the chick transport trays, whole carcass rinse samples collected on arrival at the processing plant (19%), and processing plant pre-chill carcass rinse samples (17%). *Salmonella* serotype Kentucky accounted for 52% of all isolates and appeared in 66% of the flocks in at least one sample type. *Salmonella* serotypes Typhimurium (8.4%), Montevideo (7.7%), Thompson (4.6%), Hadar (3.3%), Senftenberg (3.1%), Heidelberg (3.0%), Braenderup (2.9%), Mbandaka (2.2%), and Enteritidis (1.7%) rounded out the top ten and accounted for 36.9% of all isolates. The remaining 61 serotypes contributed a combined total of 11.1%. The greatest numbers of flocks positive for the top ten serotypes were seen in the transport tray paper samples, processing plant whole carcass rinse samples, and pre-chill carcass rinse samples. *Salmonella* serotypes Kentucky, Typhimurium, Montevideo, Heidelberg, and Braenderup were found in at least one flock at every sampling point. However, for serotypes Senftenberg, Thompson, Mbandaka, Hadar, and Enteritidis, at least one sampling point along the production continuum was negative for all 76 flocks.

Student Support: NIH grant #5T35RR007071

### **Assessing Heat Dissipation Responses in Captive Elephants to Environmental Temperature**

Chasie Johnson<sup>1</sup>, Stephanie Dray<sup>2</sup>, Andrew Smith<sup>3</sup>, Diana Barkle<sup>3</sup>, Audrey Fisher<sup>3</sup>, Andy Kouba<sup>3</sup> and Scott Willard<sup>2</sup>  
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Digital Infrared Thermal Imaging (DITI) is a non-invasive technique that has been used in the diagnosis of disease, developmental, and injury-related conditions in livestock and other species. This technique identifies thermal asymmetry of body surface temperature gradients and allows the investigator to quantify changes in skin surface temperature through detection of infrared emissivity. Elephants, like other large mammals, may face thermoregulatory issues due to the large amounts of heat their body produces, especially in warm environments. It has been suggested by others that "ear flapping" plays a major role in reducing heat load in the elephant in response to environmental stimuli. The objective of the present investigation was to observe African Elephants (n=2) at the Memphis Zoo and acquire thermal measurements of the skin surface to investigate heat exchange by the elephant in relation to ear flapping and to determine the degree to which ear flapping contributes to the regulation of body temperature. Thermal images were acquired at approximately 0600-0700, 1000-1100, 1200-1300, 1600-1700 and 1800-1900 h on each sampling day along with measures of ambient temperature (AMBT), relative humidity (RH), temperature-humidity index (THI), wind speed (WIND), and the number of ear flaps (10 min observation windows) over 12 independent days during a 1 month period. These data were categorized into AM, Early PM (EPM; 1200-1500 h) and Late PM (LPM; 1600 to 1900 h) measures for data analysis. Elephant subject characteristics were as follows: ASALI: pregnant female, 22 yr old, 3,347.51 kg, 2.51 meters, and TY: non-pregnant female, 44 yr old, 4,599.43kg, 2.67 meters. RESULTS: AMBT increased ( $P < 0.0001$ ) from AM to EPM/LPM (25 oC and 30.83/30.61 oC, respectively), but did not differ ( $P > 0.10$ ) between EPM and LPM, whereas RH decreased throughout the day (67.3%, 53.6% and 49.6% for AM, EPM and LPM, respectively). THI similarly increased ( $P < 0.001$ ) from AM to EPM/LPM and tended to differ ( $P < 0.10$ ) between EPM and LPM. WIND varied during the day but differed between AM and EPM/LPM ( $P < 0.05/0.07$ ) and between EPM

and LPM ( $P < 0.05$ ). Ear flapping frequency differed ( $P < 0.0001$ ) between elephants with ASALI exhibiting a greater number of ear flaps than TY during observations ( $26.2 \pm 1.2$  vs.  $7.9 \pm 0.49$  ear flaps/min) at AM, EPM and LPM periods. Both elephants increased in the number of ear flaps from AM to EPM/LPM, however within elephant EPM and LPM ear flap frequencies did not differ ( $P > 0.10$ ). For both elephants, AMBT, RH and THI were highly correlated with ear flapping ( $R=0.65, -0.71, 0.50$  and  $0.56, -0.44, 0.49$  for ASALI and TY, respectively), whereas WIND exhibited no relationship ( $P > 0.10$ ) to ear flapping ( $R=0.16$  and  $0.08$  for ASALI and TY, respectively). These data demonstrate variations in ear flapping responses to environmental temperatures (higher for ASALI than for TY), however show a consistent response between elephants to time of day and a lack of a response to WIND; although WIND was never greater than 1.52 m/s during the observations in this study. Additional data analysis regarding thermal imagery and relationships to environmental temperatures and ear flapping responses are currently being analyzed.

Additional data include descriptive observations of evaporative cooling post-wetting and observations of night-time thermal status for comparison to daytime measures. Overall, these data will contribute to a greater understanding of elephant physiology and adaptive cooling mechanisms in response to environmental conditions for potential application to both captive and free-ranging elephants.

Student Support: NIH grant #5T35RR007071 Research Support: USDA-ARS funded Biophotonics Initiative: #58-6402-3-0120

### **Effects of Phytoestrogens on Uterine Epithelial Growth & Gene Expression in Neonatal Gilts**

N. M. Kirk<sup>1</sup>, J.N. Feugang<sup>2</sup>, E.L. Schenck<sup>2</sup>, B. A. Bobo<sup>1</sup>, P.L. Ryan<sup>1,2</sup> <sup>1</sup>College of Veterinary Medicine and <sup>2</sup>Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS

Phytoestrogens are known to be found in high concentrations in soybean-based diets (i.e., soy-based infant formula) and a concern has arisen regarding their potential effects, both *in utero* and neonatally on reproductive development. The objective of this study was to determine whether dietary phytoestrogen exposure results in developmental aberrations of uterine tissue morphogenesis. To this end, sows were randomly assigned to a lactation diet supplemented with ( $n = 8$ ) or without ( $n = 8$ ) an isoflavone extract (Novasoy 70%, 1.5 mg/kg BW/d) from d 100 of gestation to postnatal day (PND) 14. On PND 7, female piglets were randomly assigned to one of four treatments ( $n = 4$ /treatment): 0 (CNT), 3 (LG), or 9 (HG) mg genistein/kg BW/d or 50  $\mu$ g estradiol (E2)/kg BW/d. Genistein was administered by oral gavage 2X/d for 7 days. At PND 14, piglets were weighed, euthanized and uteri were collected for histology (luminal epithelial height) and gene expression (relaxin receptor 1 and 2; RXFP 1 and 2) analyses. Data were analyzed using GLM Procedures of SAS. Uterine epithelial height was similar across groups ( $p > 0.1$ ). However, LG piglets from Novasoy-treated sows had a greater epithelial height compared to CNT ( $p = 0.08$ ) or LG ( $p = 0.07$ ) piglets from non-treated sows. Real time PCR analysis showed that uterine RXFP2 but not RXFP-1 gene expression was greater ( $p < 0.05$ ) in E2-treated piglets from non-treated sows compared to LG and HG-treated piglets from Novasoy-treated sows, and CNT piglets from non-treated sows. Further studies are needed to evaluate the interactions of phytoestrogen exposure *in utero* and postnatal exposure of estradiol or genistein on other genes (i.e., Hoxa 10, Wnta 4) associated with reproductive development.

Student Support: Merck Merial Scholar Program Research Support: USDA-ARS Biophotonics Initiative #58-6402-3-0120

### **Development of Bovine Embryos *in vitro* and Comparative Functional Genomics of Integrin Beta 5**

Koenig, Leslie; Bridges, Susan; Lawrence, Mark; and Memili, Erdogan. College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Preimplantation embryogenesis is the most critical phase of early mammalian development, setting the stage for later growth and future offspring. The objectives of this study were to determine the development and cleavage rate of bovine embryos *in vitro*, compare expression levels of Integrin Beta 5 (ITGB5) in oocyte and early embryos, and to identify comparative functional genomics of the ITGB5 protein. First, bovine embryos were

cultured in two different oxygen concentrations (20% and 5%) after which the cleavage rate and blastocyst number were determined. Both were shown to be significantly higher in 20% O<sub>2</sub> compared to 5% O<sub>2</sub>. Levels of ITGB5 transcripts were determined in MII oocytes, 2cell embryos, 8-16 cell embryos, morula, and blastocysts using real time PCR. Expression levels of ITGB5 were highest in the 2-cell embryos, followed by 8-16 cell embryos. This suggests that ITGB5 may play a role in sperm-oocyte interaction and early embryogenesis. Bioinformatics tools were used to compare ITGB5 in bovine, human, and mouse. The percent identity scores were 91.9% for bovine vs. human, 90.6% for bovine vs. mouse, and 91.7% for mouse vs. human species. Several outliers were used to create a phylogenetic tree, and the percent identity scores ranged from 68.6% for bovine vs. frog to 91.5% for bovine vs. rhesus monkey. Conserved domains occurring across all species included: vWFA superfamily, integrin beta tail, and integrin beta cytoplasmic domain. Given its very conserved nature and high percent similarity among species, ITGB5 appears to be a protein of interest in the process of fertilization.

Student Support: Merck Merial Scholar Program

### **Acetylcholinesterase Inhibition and Urinary Metabolite Levels following Repeated Exposures to Two Organophosphorus Insecticides, Chlorpyrifos and Diazinon, in Rats**

K.H. Lednum, R.H. Coombes, W.S. Bennett, E.C. Meek, M.K. Davis, J.E. Chambers. Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Organophosphate (OP) insecticides are highly prevalent in today's society. The National Health and Nutrition Examination Surveys revealed the presence of urinary OP insecticide metabolites in large numbers of random survey participants. Food and surface residues are probably the primary sources of exposure for most people. OP insecticides inhibit nervous system acetylcholinesterase (AChE) in target insects and in mammals. The inhibition of acetylcholinesterase results in the buildup of acetylcholine within cholinergic synapses and neuromuscular junctions, leading to hypercholinergic activity. A large enough exposure of OP insecticide may result in death by respiratory failure. People are normally exposed to multiple OP insecticides repeatedly. A reverse dosimetry mathematical model could predict the exposure scenario through the use of biomarkers such as urinary metabolites or blood AChE inhibition data. These experiments were designed to acquire the biomarker data that could be used for subsequent model development. Rats were exposed to chlorpyrifos (CPS) and diazinon (DZN) alone and in combination on 4 occasions over a 15 day period, and brain, plasma and red blood cell AChE and CPS and DZN urinary metabolites, 3,5,6-trichloro-2-pyridinol (TCP) and 2-isopropyl-4-methyl-6hydroxypyrimidine (IMHP), respectively, were monitored. AChE inhibition was measured in brain or blood preparations spectrophotometrically. AChE inhibition was dose related, with inhibition in the plasma and RBC's greater than in the brain. Partial recovery of AChE activity occurred between exposures. Increased inhibition with repeated exposures was relatively greater in brain than in blood. TCP and IMHP were quantified in acid-hydrolyzed urine extracts using gas chromatography with electron capture (TCP) or nitrogen-phosphorus (IMHP) detection. The highest levels of metabolites were observed on the days following treatments. When complete, these data sets will be used to calibrate the reverse dosimetry model.

Student Support: NIH grant #5T35RR007071 Research Support: EPA STAR grant RD 83345101

### **Nurr1 mRNA expression in dopamine neurons isolated with laser capture microdissection**

Talisha Moore, Jeffrey Eells Mississippi State University, College of Veterinary Medicine, Department of Basic Science Mississippi State, MS

Previous research ascertained that Nurr1, an inducible transcription factor, is imperative in development, maturation, and survival of ventral mesencephalic dopaminergic neurons. Additional research implicated several dopamine (DA) neurotransmission genes as target genes of Nurr1 as Nurr1-null heterozygous mice have attenuated expression of some of these genes. Nurr1 expression can oscillate in response to alterations in neuronal activity and stimulation of DA autoreceptors, however, the contribution of these mechanisms on Nurr1 expression is unclear. The goal of the current project is to determine the factors affecting Nurr1 expression in dopamine neurons. To that end, reverse microdialysis of the dopamine D<sub>2</sub> receptor antagonist eticlopride was

infused into the substantia nigra of adult male rats for 90 min. Microdialysis samples were measured for DA release using HPLC with electrochemical detection. Ongoing results suggest a trend towards elevated DA release after eticlopride treatment. Laser capture microdissection (LCM) will be used in combination with tyrosine hydroxylase immunocytochemistry to isolate 100+ DA neurons from the substantia nigra. We have developed methods to measure small amounts of RNA (down to 10 pg/0l) from these cells using the Nanodrop 3300 and Ribogreen fluorescence and, using RNA isolated from whole sections, to determine the quality of the RNA on the slide using the Bioanalyzer 2100 and the RNA 6000 pico labchip. Although research is ongoing, we hope that the combination of these techniques and equipment will reveal answers to the cellular and molecular factors that affect diseases such as schizophrenia and Parkinson, both of which are affected by dopaminergic neuron dysfunction.

Student Support: MSU College of Veterinary Medicine

### **The Impact of Water Quality on Broiler Performance**

Virginia Munson\*1, Phil Stayer<sup>2</sup>, Mark Burleson<sup>2</sup>, and Chinling Wang<sup>1</sup> College of Veterinary Medicine, Mississippi State University<sup>1</sup>, and Sanderson Farms<sup>2</sup>.

At normal temperatures, chickens consume twice as much water as feed. Water is a vital nutrient that impacts virtually every physiological function in the body. Chemical characteristics can impede digestion or absorption of additives like medicines, vaccines, or vitamins. In this way, water has a big impact on both the health status and the production results of the birds. The objective of this study was to determine the relationship between well water quality and flock performance in commercial broilers. A total of 52 water wells at broiler farms centered around McComb production were chosen. The water was tested for calcium, magnesium, sodium, potassium, boron, carbonate, bicarbonate, sulfate, chloride, nitrate, phosphorous, pH, conductivity, hardness, alkalinity, total dissolved solids, sodium absorption ratio, iron, zinc, copper, and manganese. The performance of the flocks using these 52 water wells was then assessed based on the average of 10 flocks per house. The livability, condemnations, and deviation from standard price were used to assess the overall performance of the birds. The results indicated that the highest livability farms had all minerals below the recommended levels while the lowest livability farms had higher values of potassium, sodium, carbonate, sulfate, phosphorous, alkalinity, and total dissolved solids. Most farms with low condemnations had low mineral content, while the farms with average and elevated condemnations had higher mineral content in their water. However, there was no relationship between water quality and flock performance. This study suggests that mineral contents in the well water have an impact on the broiler livability and condemnation, but not necessarily flock efficiency.

Student Support: Merck Merial Scholar Program

### **Evaluation of effects of freezing on *Salmonella* enumeration in broiler carcass rinses**

Jennifer Sims, R.H. Bailey, and R.W. Wills College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

*Salmonella*, a member of the family *Enterobacteriaceae*, is a gram-negative bacteria that causes enteric disease in multiple animal species. The poultry industry faces increased regulatory pressure concerning the presence of *Salmonella* in the processing environment and scientifically sound risk factor information is needed to make logical risk management decisions pertaining to microbial control. The development of techniques to do risk factor analysis in the production environment requires the use of methods that are the most sensitive and accurate for both sampling and culturing of *Salmonella*. A primary focus of our laboratory is to fully characterize the ecology of the immersion chill tank in order to optimize its operation in controlling *Salmonella* contamination in the processing of broilers. The purpose of this specific work was to determine the most appropriate method for disposition of carcass rinse samples prior to culturing using the Most Probable Number Modified Secondary Enrichment Method. The effect of freezing on *Salmonella* numbers prior to culturing was determined using both spiked and naturally contaminated samples. The information obtained in this work is a critical component of a larger project which is evaluating the association of current *Salmonella* control measures used by the broiler industry with specific parameters, including differences in *Salmonella* contamination levels on the birds prior to

entering the immersion chill tank, and the interrelationship of pH, chlorine levels, turbidity, water flow rate, and/or temperature in the water used in the chill-tank.

Student Support: Merck Merial Scholar Program

### **An Assay to Quantify Neutralizing Antibodies in Amniotic Fluid from FIV-Infected Cats**

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The feline immunodeficiency virus (FIV)-infected cat serves as an excellent small-animal model for HIV pathogenesis, because infected cats develop a syndrome that closely resembles human AIDS. In addition, FIV can be transmitted across the placenta, producing an infected fetus and/or reproductive failure, conditions that are well documented globally in HIV-infected women. Infected amniotic fluid (AF) is a potential source of fetal infection. Therefore, virus-neutralizing antibodies, which can be present in AF, may play a role in limiting fetal infection. The purpose of this study was to quantify FIV-neutralizing antibodies in paired AFs and sera collected from FIV-infected queens. Fetuses were delivered from three FIV-infected queens by cesarean section at week 3-4 of gestation. Sera were collected from each queen, and AFs were aseptically aspirated from individual gestational sacs and cryopreserved until needed. The neutralization assay was performed by incubating serially-diluted AFs or sera with FIV-B-2542 in 24well culture plates, followed by the addition of MCH 5-4 cells, a feline T cell line. Triplicate cultures, along with appropriate controls, were incubated for 1 week. DNA was harvested from all samples for use as a template in PCR targeting a 293 bp region of the FIV gag gene to determine whether the cells harbored provirus. PCR analyses are currently ongoing and results are pending. Cell culture supernatants will be analyzed at a later date for viral antigen.

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### **Role of Internalins in *Listeria monocytogenes* Invasion of Intestinal Epithelial Cell Lines**

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*Listeria monocytogenes* is a gram positive bacterium that can be isolated from the soil, water, and other environments. It is an important food borne pathogen that causes listeriosis in humans and animals. The common symptoms in humans are gastroenteritis, meningitis, encephalitis, and sepsis. In pregnant women miscarriages as well as premature delivery of the unborn can occur. *L. monocytogenes* has been isolated from many ready-to-eat products, including those in the aquaculture industry. Cell culture models with the human cell line Caco-2 (colorectal adenocarcinoma) are used to evaluate the pathogenic potential of *L. monocytogenes* isolates and determine their ability to invade mammalian cells. The listerial proteins that mediate this ability to invade are termed internalins, and they are unique to the genus *Listeria*. This study had two major objectives. First, we determined if the guinea pig colorectal adenocarcinoma cell line, GPC-16, would be an effective, safe alternative to using the human cell line Caco-2 to evaluate invasion and virulence. Caco-2 and GPC-16 cells were grown in culture and then infected with strains F2365, EGD, and HCC23. Second, we determined if internalins expressed by some strains of *L. monocytogenes* are responsible for pathogenicity and the ability to invade cells. The HCC23 strain of *L. monocytogenes* is nonpathogenic and does not possess the internalins Lmo2470, Lmo2821 (InIJ), and Lmo1812 (InIC). These internalins were amplified, cloned, and transferred into the chromosome of HCC23.

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### **Detection of tick-borne agents from *Amblyomma americanum* (lone star tick) in Mississippi**

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*Amblyomma americanum* (lone star tick) is a common tick in the southeast. Within its range, it is a vector for human disease agents such as *Borrelia lonestari*, putative agent of “southern tick-associated rash illness” (STARI) and *Ehrlichia chaffeensis*, agent of human monocytic ehrlichiosis. The purpose of this study was to evaluate the prevalence of bacterial agents in lone star ticks in Mississippi. Over 700 lone star ticks from four regions of Mississippi: Northeast, Northwest, Southeast, and East were collected for this project. Of the ticks collected, 192 were dissected and the DNA was extracted for PCR assays. To confirm the presence of tick DNA, we first amplified the tick mitochondrial 16S rRNA gene; all extracted ticks were PCR positive. A species-specific nested PCR technique was performed for each of the ticks to test for *Borrelia* sp. and *Ehrlichia chaffeensis*. Thus far, of the ticks tested, 2 out of 160 had evidence of *Borrelia* sp. DNA. These positive samples are being sequenced to identify *Borrelia* species. 1 out of 100 of the ticks was PCR positive for *Ehrlichia chaffeensis*. In addition to testing ticks by PCR assay, we also cultured 20 ticks from two locations in an attempt to grow these bacteria. Cultures are currently growing and will be PCR assayed to detect organism. These studies support the presence of *Borrelia* species in Mississippi and may demonstrate the presence of other tick-borne bacteria as well.

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### **An Evaluation of Physical and Behavioral Signs of Pain in Large Felids**

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Pain management is a concept that has become increasingly important in the veterinary community over the last decade. However there is a need for research on the differences in manifestations of pain and distress in large cats versus domestic cats. Large cats may have inherent genetic and behavioral differences that cause them to physically and behaviorally manifest pain in different ways than their domestic cousins. This study employed a two phase design aimed at documenting normal behavior in captive tigers at a rescue facility as well as on displays at multiple zoos. Animals were observed and videotaped at set times. Behaviors were then charted and graphed to establish frequencies and trends. An ethogram was created for all of the animals observed. There was a high number of uncontrollable variables such as the age and history of the subjects, but our study revealed important trends that had not been previously documented. Subject's showed an unusual amount of socialization considering the species. Tigers in the wild are solitary animals yet most of the captive subjects in our study were in group settings. Surprisingly the animals that were not housed in pairs displayed a higher frequency of anxiety driven behaviors (pacing, spraying, etc.). Data still needs to be validated for significance.

The second phase of the study will be aimed at recognizing abnormal behaviors in response to noxious stimuli (stress, pain). The long term goal is to establish a scaling system like the Glasgow pain scale that can be applied to evaluations of large cats.

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