Student Abstracts 2011

Front (L to R): Ashley Adams, Angela Free, Rachel Smith, Susan Rodgers, Ashlee Oliver

2nd row: Samantha Vitale, Hadley Harris, Jenica Haraschak, Lauren Bright, Tracy Thompson, Lauren Phillips, Crystal Hall, Steven Davison

3rd row: Michael Orencole, Dr. Jeff Eells, Darin Kepler, Ryan Bear, Devon Livingston, Brooklynn LaFoon, Dr. Mark Lawrence

Effect of Repeated Developmental Low Dose Exposure to Chlorpyrifos on Anandamide Hydrolysis in the Juvenile Rat Brain. Ashley L. Adams* and Russell L. Carr

The endogenous cannabinoid arachidonyl ethanolamide (AEA or anandamide) plays a vital role during nervous system development including regulating axonal guidance and synaptogenesis. The enzyme, fatty acid amide hydrolase (FAAH) is responsible for the hydrolysis of AEA and is susceptible to inhibition by organophosphate compounds *in vitro*. Furthermore, acute *in vivo* exposure of adult animals to the agricultural insecticide chlorpyrifos (CPS) caused moderate inhibition of AEA hydrolysis. However, the effects of repeated exposure to lower levels of CPS, especially during times of development, on endocannabinoid metabolism in the brain are not known. To examine this, rat pups were orally exposed daily from postnatal days (PN D) 10-16 to 1.0, 2.5 or 5.0 mg/kg CPS and sampled at 4, 12, and 24 hours following the last administration on PND16. Body weight gain was reduced on all days of treatment with 5.0 mg/kg CPS and on the last two days of treatment with 2.5 mg/kg.

No reduction in weight gain was observed with 1.0 mg/kg. At 4 hours, forebrain cholinesterase (ChE) activity and AEA hydrolysis were inhibited in a dose related manner. At 4 hours, the extent of inhibition of forebrain AEA hydrolysis was approximately twice that of ChE with AEA hydrolysis being virtually eliminated by 2.5 mg/kg and 5.0 mg/kg CPS while 1.0 mg/kg CPS caused 40% inhibition. Little recovery of activity had occurred by 12 hrs whereas 24 hrs, some recovery was observed but hydrolysis was still significantly inhibited. The levels of AEA in the forebrain were significantly elevated at 12 hours with some recovery by 24 hours. The increased sensitivity of AEA hydrolysis suggests its potential as an alternative developmental target for CPS rather than the traditionally accepted inhibition of ChE. The observed increased accumulation of AEA induced by inhibition of its hydrolysis could be detrimental to brain maturation.

Student Support: NIH Grant #5T35RR007071

Potency of a Covalent Anthracycline-[Anti-HER2/neu] Immunochemotherapeutic Against Mammary Carcinoma Combined with Benzimidazole Tubulin Inhibitors. Ryan Bear*, C. P. Coyne, and T. Jones
In an effort to improve efficacy against aggressive chemotherapeutic resistant mammary carcinoma and reduce the side effects associated with conventional chemotherapy, the anthracycline epirubicin was covalently linked to anti-HER2 monoclonal immunoglobulin. The synthetic methodology involved the application of reagents at the a-monoamide group of epirubicin to create a reactive intermediate. A second stage of synthesis was conducted by crosslinking the reactive intermediate to immunoglobulin at the terminal amine position of e-lysine amino acid residues. The epirubicin-(C3 amide)-[anti-HER2/neu] immunochemotherapeutic agents exhibited a high degree of cell binding while maintaining the desired anti-neoplastic effect of the anthracycline chemotheraputic against a chemotherapeutic-resistant SKBr-3 mammary carcinoma cell line. In a parallel study, alternative benzimadazole tubulin/microtubule inhibitor chemotheraputic agents were applied against the same chemotheraputic resistant SKBr-3 strains of mammary carcinoma resulting in anti-neoplastic activity that has yet to be described in the study of mammary carcinoma. Application of the epirubicin-(C3 amide)-[anti-HER2/neu] immunochemotherapeutics in concert with the tubulin inhibition chemotherapeutics mebendazole and griseofulvin enhanced the potency of the immunochemotheraputics. By presenting a novel synthesis method for covalently binding anthracyclines to monoclonal immunoglobulins and establishing the potential for tubulin inhibitors to additively or synergistically enhance the potency of conventional chemotherapeutics, these investigations present a model suggestive of potential strategies that could enhance anti-neoplastic potency of chemotherapeutics in other multi-drug resistant neoplasias.

Student Support: Merial Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine

Proteomic Analysis of Bronchoalveolar Lavage Fluid from Horses with Summer Pasture Associated Recurrent Airway Obstruction Identifies Changes in Neutrophil Regulation. Lauren A. Bright*, Fiona M. McCarthy, Bindu Nanduri, Leslie A. Shack, Nisma Mujahid, Lais R. R. Costa, Shane C. Burgess, and Cyprianna E. Swiderski
Equine summer pasture-associated recurrent airway obstruction (SPARAO) is a seasonal, environmentally induced severe respiratory disease with prominent airway neutrophilia. This airway neutrophilia was once thought to distinguish SPARAO from human asthma, but airway neutrophilia is now recognized in over half of human asthmatics and correlates to disease severity. Building upon our prior evidence that protein functions in normal lung fluids are conserved across horse, human, and mouse, this project aims to characterize molecular events mediating airway neutrophilia in SPARAO. Using shotgun proteomics, we identified differentially expressed proteins in bronchoalveolar lavage fluid (BALF) of affected and control horses and modeled the effects of these proteins on neutrophil function. Pooled cell-free BALF collected from 6 SPARAO affected and 6 nonaffected herdmates during seasonal clinical exacerbation were subjected to 1D liquid chromatography nanospray tandem mass spectrometry. Proteins were identified and quantified using SEQUEST and

PROTQUANT, respectively. The role of these proteins in neutrophil function was contrasted using Ingenuity Pathway Analysis®, and assigned a numerical score according to the proteins' role as pro- (+1), anti-(-1), or no effect (0). Contributions of each protein to the neutrophil functions were calculated as the product of magnitude of protein expression multiplied by its effect.1003 proteins were identified ($P<0.05$; 472 unique to nonaffected, 417 unique to affected, and 114 proteins common to both groups). Relative to control horses, proteins in the BALF of affected horses upregulate cell movement, chemotaxis, and activation, and reduce transmigration, detachment, migration, apoptosis, and neutrophil infiltration. This data demonstrates the utility of systems modeling to organize functional genomic datasets in order to characterize complex molecular events associated with clinically relevant equine disease.

Student Support: Morris Animal Foundation Veterinary Student Scholars Program and Mississippi State University College of Veterinary Medicine

A Study of Infected Canine Wounds: Bacteriology and Antimicrobial Susceptibility (222 Isolates from 2003-2011). Jeffrey F. Comstock*, Jennifer L. Wardlaw, and Frank W. Austin
The objectives of this study were to identify the most common canine wound bacteria, and assess trends in drug resistance for the most common pathogens, in an effort to better guide clinical therapy. Medical records were reviewed for signalment, culture date, reason for presentation, culture site, previous antibiotics, number of bacterial pathogens in the wound, the number of serial cultures, and antimicrobial resistance profile of each isolate. Patients were omitted if they had a negative culture. A total of 135 cases with 222 isolates were obtained. Patients were represented by males (31%), neutered males (29%), females (17%), and spayed females (22%) with an average age of 4.8 years (range 3 months-14 years). The reasons for presentation were a wound (34%), hit-by-car trauma (25%), postoperative issues (17%), dog fight (14%), and open fractures (10%). The type of wounds were dermal (56%), penetrating (20%), incisions (13%), and abscesses (12%). The most common bacterial genera were; *Staphylococcus* (48%), *Pseudomonas* (19%), *E. coli* (16%), *Enterococcus*(13%), and *Streptococcus* (9%). *Staphylococcus* and *E. coli* had both resistant strains and more susceptible community strains. *Pseudomonas* and *Enterococcus* were all multiple drug resistant (MDR) strains. *Streptococcus* was readily susceptible to most antibiotics. Antibiotics given before the culture was more likely to result in the presence of the resistant *Pseudomonas* ($p=0.02$). Deeper, penetrating wounds did not contain *Enterococcus*. During the last four years, compared to the beginning of the study, the non-MDR *Staphylococcus* strains have become susceptible to ampicillin whereas the MDR*Staphylococcus* strains have developed resistance to gentamicin. *Enterococcus* had less than 80% susceptibility to all profiled antimicrobials. Findings suggest a change in the resistance profile for*Staphylococcus* and the previously unreported appearance of *Enterococcus* in wounds, but no increase in the prevalence of MDR infections over the course of this study.

Student Support: NIH Grant #5T35RR007071

PTX3 in the Rabbit Model of Hypertension and Aortic Valve Stenosis. S. Davison*, E. Brinkman-Ferguson, A. Claude, D. Dycus, D. Lodato, R. Cooper, and J. Warnock
Plasma levels of Pentraxin 3(PTX3), a member of the long pentraxin family, are increased in human patients with aortic valve stenosis, but physiology and function of PTX3 is poorly understood. The objective of this study was to better understand the role of PTX3 as a possible diagnostic and prognostic indicator as well as a treatment target. It was hypothesized that plasma levels of PTX3 would be increased in a rabbit model of aortic valve stenosis, but not increased significantly in sham models. Nine adult male New Zealand White rabbits were used in this study; five rabbits underwent Goldblatt one-kidney/one-clip surgery to induce hypertension and aortic valve stenosis and four sham models served as controls. Plasma levels of PTX3 were measured in all rabbits before surgery and two and four months after the procedure. Additionally, isolated porcine aortic valve interstitial cells were exposed to Angiotensin II (300nM), TNF-α (10 ng/mL) or cyclic pressure (120/80 mmHg) for six hours. This was done to determine the main stimuli for PTX3 expression in the pathogenesis of aortic valve stenosis. Rabbits that underwent Goldblatt surgery showed increased blood pressure [128/112 mmHg compared to 92/87

mmHg before surgery(n=2)] after two months. Cell culture studies showed that cyclic pressure increased PTX3 gene expression (1.4 fold increase compared to control) and levels of PTX3 protein in the culture supernatant (21 pg/mL increase compared to control). Angiotensin II and TNF-α did not cause any significant change in PTX3 gene or protein expression. In conclusion, preliminary data suggests that cyclic pressure drives PTX3 expression implying that gene and protein expression are mechanosensitive.

Student Support: Merial Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine

Survival of O157:H7 and non-O157:H7 shiga-toxin producing *Escherichia coli* in bovine bile salts and rumen fluid. Angela L. Free*, Heather A. Duoss, Ty B. Schmidt, and Janet R. Donaldson

Escherichia coli are gram negative, facultative anaerobic bacteria that colonize within the intestines of animals and humans. Enterohemorragic strains of *E. coli*, specifically those that produce shiga-toxin (STEC), pose a serious health risk to humans yet reside asymptomatically within ruminants. In particular, bovine serve as the major reservoir for these bacteria. STEC research has historically been focused upon O157:H7. However, with an increase in food-borne outbreaks of non-O157 origin, there is now a critical need to understand the biology behind these other serotypes as well. The focus of this study was to determine whether variations exist in the ability of different serotypes of STEC to survive within bovine bile salts and rumen fluid. The results of this study indicate that the five serotypes tested (O157:H7, O111:H8, O103:k:H8, O145:H28, O26:H11) grew in rumen fluid equally well. However, variations were seen in the survival of these STEC in bovine bile salts. These data suggest that non-O157:H7 serotypes of STEC respond differently than O157:H7 to the bovine intestine. Further work is needed to decipher how these variations correlate with alterations in colonization within ruminants.

Student Support: NIH Grant #5T35RR007071

Development of a Proximity Ligation Assay to Quantify Influenza-Antibody Interaction. Crystal Hall* and Xiu-Feng (Henry) Wan

Influenza A viruses have caused large losses of lives around the world and continue to present a great public health challenge. The mutations in HA and NA cause antigenic drift since these two genes are the primary targets for host immune systems, and these mutations can result in variation of viral antigenicity. These antigenic drift variants allow the virus to escape the host immunity and cause disease outbreaks and epidemics. In addition, by exchanging genomic segments between two or more different viruses, a reassortant virus can be created. Rapid detection of novel influenza virus and sensitive quantification of influenza-antibody interaction will be useful for influenza diagnosis as well as vaccine strain selection. The conventional hemagglutinin inhibition (HI) assay routinely used in influenza vaccine strain selection is a coarse assay and heavily affected by the types of red blood cells. Recently qRT-PCR based proximity ligation assay has been shown to be effective in influenza diagnosis through measuring influenza-antibody interaction using monoclonal antibody. In this study, we aim to develop and validate a proximity ligation assay to quantify influenza-antibody interaction, especially using polyclonal antibody. An H3N2 canine influenza A virus and ferret antisera were utilized to assess this sensitivity and specificity of this assay. The results and limitations are discussed.

Student Support: NIH Grant #5T35RR007071

Pharmacokinetics of Oral Dantrolene in the Dog Measured by High-Performance Liquid Chromatography. J. Haraschak*, K. Lunsford, C. Langston, A. Mackin, and T. Archer

Dantrolene is a muscle relaxant that has long been used in several species. Recent reports in the biomedical

literature have suggested that dantrolene is also a significant inhibitor of T-cell function, and may be a more potent immunosuppressant agent than currently available drugs. In the oral form, dantrolene has a relatively mild side effect profile and is more affordable than many other immunosuppressive agents. As such, dantrolene may be an important addition to the arsenal of veterinary immunosuppressive drugs. Oral administration is the preferred route of administration when treating chronic diseases in dogs. While the pharmacokinetics of intravenous dantrolene have been studied in dogs, the pharmacokinetic profile of oral dantrolene has not, and this information is critical to future studies relating to the immunosuppressive effects of the drug. Our group has recently developed novel molecular methods for the direct evaluation of T-cell function in dogs. The purpose of our current study was to obtain crucial pharmacokinetic data needed to develop future *in-vitro* and *in-vivo* studies of the effects of dantrolene on T-cells. A randomized, balanced two-way cross-over design utilizing 6 healthy dogs was employed. Half of the study population received a single oral dantrolene dose of 5 mg/kg, and the other half received 10 mg/kg. Following a 2 week washout period, the groups were reversed and received the alternate dose. Blood samples were collected for high-performance liquid chromatography analysis of dantrolene concentrations at time zero and at appropriate time points after drug administration. The pharmacokinetic parameters which were evaluated in this study included the peak plasma dantrolene concentration, time to peak plasma concentration, elimination half-life, and the oral bioavailability of dantrolene and its metabolite 5-hydroxydantrolene. Our study was the first step in exploring the utility of dantrolene as an immunosuppressive agent in dogs.

Student Support: Merial Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine

High density packaging of boar spermatozoa and identification of potential bad and good freezers. Sarah H. Harris*, Jean M. Feugang, Bayard S. Grillis, Devon A. Livingston, Scott T. Willard, and Peter L. Ryan
The widespread use of semen cryopreservation is limited in boar studs due to their poor freezability, which is affected by various factors including the boar itself. Indeed, spermatozoa of a subset of boars do not support cryopreservation well and there are no reliable criteria to predict the freezability potential of a boar. As the first step of a long-term objective, our study aimed at defining “bad” and “good” freezer sires in a herd of highly fertile boars. Semen was obtained from independent ejaculates (≥3) of five boars (Prestage Farms, MS). Spermatozoa were pelleted and diluted in the freezing solution (Lactose-Egg yolk and 3% glycerol). After equilibration (2h-4oC), 0.5ml-plastic straws were filled with 187x, 375x, and 750x 106 spermatozoa/ml and exposed to liquid nitrogen vapor (-140oC) for 30-minutes. Post-thaw motility and viability data were analyzed within 30-minutes incubation in extender at 37oC. Fresh spermatozoa were pelleted and stored for protein analysis. Data were analyzed using the student’s t-test and expressed as mean±sem. $P<0.05$ indicated significant differences. There were not significant differences in motility and viability data between 15- and 30-minutes post-thaw analyses. Regardless of boars, the proportions of motile, rapid and viable spermatozoa were decreased after freezing (20%±5%, 9%±2% and 15%±6% vs. 88%±2%, 70%±3% and 88%±2% in fresh, respectively; $p<0.05$). All sperm densities showed similar motility rates. There was a tendency for lower viability in the high-sperm density group compared to others (11%±4% vs. 17%±6%). Nevertheless, a group of three boars showed significantly higher motility (24%±3% vs. 16%±2%) and viability (18%±6% vs. 10%±1%) rates than others. Additionally, qualitative and quantitative differences were observed at the protein level between both groups. This study indicates the existence of boars with different spermatozoa cryotolerance and protein contents. Characterization of critical proteins could help either select “good freezer” boars or successfully cryopreserve “bad freezer” spermatozoa.

Student support: USDA-ARS Biophotonics initiative #58-6402-3-0120 and Mississippi State University College of Veterinary Medicine

Monoacyglycerol lipase inhibition following developmental chlorpyrifos exposure in juvenile rats. Darin R. Kepler* and Russell L. Carr

The endocannabinoid neurotransmitter system is important for normal neural development and synaptogenesis. The predominant endocannabinoid in the brain is 2-arachidonoylglycerol (2-AG) whose degradation is mediated by monoacylglycerol lipase (MAGL). In adults, acute exposure to the organophosphorus insecticide chlorpyrifos (CPS) results in the inhibition of MAGL and elevation of 2-AG in the brain. However, it is not clear if low level exposure produces similar results especially in low level repeated exposure. Given the importance of the endocannabinoid system in brain maturation, developing animals could be at greater risk to the disruption of this system. To investigate this, 10 day old rats were exposed to either 1.0, 2.5, or 5.0 mg/kg CPS for 7 days and MAGL activity was determined in the forebrain at 4, 12, and 24 hrs post-exposure on day 7. MAGL activity was inhibited in a dose dependent manner (14%, 22%, 37%) at 4 hours with slight recovery by 24 hours (roughly 5-10%). In addition, exposure also resulted in the significant elevation of 2-AG at 12 hrs and 24 hrs. However, in juveniles, MAGL was only responsible for 70% of the hydrolysis of 2-AG. Using specific inhibitors for other candidate enzymes such as fatty acid amide hydrolase (FAAH), ABHD6 and ABHD12, it was determined that ABHD12 was responsible for the roughly 9% of the activity while FAAH and ABHD6 did not contribute appreciably to total 2-AG hydrolysis. The source of the remaining ~20% of the 2-AG hydrolysis is currently unknown.

Student Support: NIH Grant #5T35RR007071

Functional Annotation of the Affymetrix Canine 2.0 Microarray. Brooklynn LaFoon*, Kriston Weaver, Andrew Mackin, Lesya Pinchuk, and Fiona McCarthy

Lack of functional annotation associated with arrays hinders researchers who wish to model their array data to obtain biologically relevant insights. The most commonly used canine array is the Affymetrix GeneChip ® Canine Genome 2.0, which has probes available for18,000 canine gene products. In order to facilitate canine functional modeling, we reannotated the Affymetrix canine array since the public database accessions and functional annotation associated with this array were last updated five years ago in 2006. We used primary sequence identifiers and mapped these to other public databases to obtain the most recent ID mappings for transcripts represented on the array. This updated data will enable researchers to more efficiently retrieve available information about their data sets. Next we linked these identifiers to link to existing functional annotation from the Gene Ontology (GO). When there was no available GO we manually biocurated published literature where it was available or did sequence analysis when literature was not available. Prior to ID remapping, the canine array had 15,822 annotations for 2,608 gene products. After remapping these IDs, we identified 10,737 existing GO annotations for 5,127 of the newly mapped gene products represented on the array. In addition, we added 70,148 annotations for 16,966 gene products. In conclusion, we have been able to provide annotations for 94% of this array, and this represents a fourfold increase in the number of gene products that were initially annotated. The updated array annotation that will be publicly available at AgBase, can be incorporated into tools that utilize functional data for modeling, and just as importantly, will provide the basis for functional data for other canine arrays and functional genomics data sets.

Student Support: NIH Grant #5T35RR007071

High density packaging of equine spermatozoa and identification of potential bad and good freezers. Devon A. Livingston*, Jean M. Feugang, Bayard S. Grillis, Abed Fahad, Sarah H. Harris, Scott T. Willard, and Peter L. Ryan

Equine spermatozoa are highly sensitive to cryopreservation and not all stallion semen freezes alike. Unfortunately, there are no reliable markers to predict the freezability status of stallions, which would enhance the widespread application of cryopreservation in the equine industry. The present study aimed at discriminating potential “bad” and “good” freezer stallions in a herd maintained at Leveck Animal Research Center of Mississippi State University. Semen was obtained from independent ejaculates (≥3) of four stallions and diluted in INRA-82 extender. Spermatozoa were pelleted and diluted with freezing solution (INRA-82 plus 2.5% glycerol).

After equilibration (2h-4oC), 0.5ml-plastic straws were loaded with 4x, 8x or 16x108 spermatozoa/ml, immediately frozen at 6oC/min from 4oC to -150oC (Crysalys CryoController, Biogenics) and stored at -196oC until use. Motility and viability parameters were analyzed within 30 minutes incubation in INRA-82 extender at 37oC. Fresh spermatozoa were pelleted and stored for protein analysis. Data was analyzed using the Student's t-test and expressed as mean±sem. $P<0.05$ indicated significant differences. The motility and viability data were similar at 15- and 30-minutes post-thaw. For all stallions, the percentage of motile, rapid and viable spermatozoa was decreased after freezing (56%±6%, 23%±3% and 51%±13% vs. 82%±5%, 61%±5% and 85%±1.3% in fresh, respectively; $p<0.05$). The highest sperm density per straw exhibited the lowest results compared to other groups, which were not significantly different (motility: 18%±4% vs. 54%±7% and viability: 35%±10% vs. 57%±9; $p<0.05$). Interestingly, two stallions showed higher post-thaw motility and viability (64.7%±0.4% and 54%±9% vs. 40.8%±2.2% and 48%±15%; $p\leq0.05$). Both stallions also displayed qualitative and quantitative protein differences with others. This study indicates the possibility of using controlled-slow freezer systems in stallions and increasing the sperm density per freezing straw. Furthermore, the characterization of specific proteins in cryotolerant spermatozoa could lead to the development of novel prognostic markers for identifying "good" freezer stallions.

Student Support: NIH Grant #5T35RR007071

Evidence of selective packaging and different α-granule subtypes on canine platelets. Sara Minear*, Sandra Curotto, Whitney Smith, Kristine von Maltzan, Kari Lunsford, and Camilo Bulla

The balance of pro and anti-angiogenic factors present in platelets has been shown to be predictive of tumor growth. Platelets are able to take up both vascular endothelial growth factor (VEGF), and endostatin, a strong anti-angiogenic molecule. During development, platelets are able to selectively package molecules within different α-granule subsets, and similarly molecules taken up by circulating platelets can also be selectively sorted. Moreover, selective α-granule release upon platelet activation is dependent on specific receptor activation. Although the evidence points to an intimate relationship between endothelial cells and platelets with respect to pathologic tumor angiogenesis, the specific signaling mechanisms involved remain unknown. Numerous studies implicating the platelet in the regulation of tumor angiogenesis and demonstrating its potential as a novel source for biomarkers of tumor growth have been carried out in rodent models, however little work has been performed with naturally occurring disease. In order to better understand platelet-tumor relationships, a naturally occurring disease model is desirable. As such, we are evaluating the dog as a suitable and, in fact, superior model for human platelet-tumor interactions *in vivo* and the impact of these interactions in naturally occurring disease. Here, we undertook an initial step in testing the hypothesis that differential packaging of proteins in distinct α-granule subtypes occurs in dog platelets. Platelet rich plasma was obtained from parafolmaldehyde fixed citrated blood and platelets were adhered to a slide by cytospin. Sheep polyclonal IgG anti-canine vWF and mouse monoclonal IgM anti-canine fibrin/fibrinogen antibodies were used for dual immunolabeling. Images were acquired using a Zeiss LSM 510 confocal laser scanning microscope with an inverted Zeiss Axiovert 200 M light. Different localization of fibrinogen and vWF in the cytoplasm of canine platelets was clearly visualized, indicating that dogs share selective packaging on α-granule ontogeny, and as humans have different α-granule subtypes.

Student Support: NIH Grant #5T35RR007071

Creation and Characterization of *Listeria monocytogenes* mutants. Ashlee Oliver*, Swetha Reddy, Jennifer Nichols, Michelle M. Banes, andMark L. Lawrence

Listeria monocytogenes is a facultative anaerobic gram-positive bacterium that can propagate at refrigeration temperatures. Itis the causative agent of listeriosis and a leading cause of death among known foodborne pathogens. Previous research on the genome sequences of high-risk serotypes (4b strain F2365; 1/2a strain EGD-e) compared with low-risk serotypes (4a strain HCC23 and *Listeria innocua*) revealed 58 genes shared by the pathogenic serotypes that were absent in the non-pathogenic serotypes. In this investigation, we worked on

construction of mutants for eight pathogenicity associated genes. For each of the eight genes, overlap extension PCR was used to amplify a gene deletion from F2365 that was first inserted into plasmid pGEM-T (all eight genes successfully inserted) and then subcloned into shuttle vector pAUL-A (*lmof2365_0281* and*lmof2365_0088*,which code for internalin C and a hypothetical protein, were successfully inserted). Electroporation and temperature shifts were used to integrate the gene deletions into the genome of *L. monocytogenes*. A previously created mutant *lmof2365_2464* (an HD domain-containing putative hydrolase) was partially characterized using adhesion and invasion assays with HT-29 epithelial cell line. The *lmof2365_2464* deletion mutant was able to adhere to HT-29 cells as effectively as the wild type strain. However, the mutant strain was far less effective in invading the HT-29 cells than the wild type. This could indicate that *lmof2365_2464* is required for effective uptake and/or replication in enterocytes.

Student Support: NIH Grant #5T35RR007071

Motor cortex white matter asymmetry in the cat as determined by diffusion tensor imaging. Michael J. Orencole*, Ruiyi Wu, Robert E. Meyer, David F. Tate, Song Zhang, and Jeffrey B. Eells
Diffusion tensor imaging (DTI) enables the visualization of white matter fasiculi in both two and three dimensions. DTI provides a noninvasive, comprehensive anatomical map of the white matter allowing for the study of white matter differences in pathological conditions as well as in normal brain function. Hemispheric lateralized asymmetrical differences associated with handedness in humans have been demonstrated in motor pathways. Paw preference in cats have also been demonstrated based on a complex motor task. Surprisingly, this lateralized behavior was strongly sex related with females showing a strong preference for the use of their right paw and males showing a strong preference for the use of their left paw. Based on these data, our hypothesis was that fiber tract asymmetries in the corticospinal pathway controlling the front limb would be found in cats using DTI and would be related to sex. To test this hypothesis, a GE 3T MRI machine was used to image 5 cats (3 females and 2 males). Brodmann's areas 4 and 6 along with the internal capsule were targeted as our regions of interest (ROI) and then compared bilaterally to provide a 3D map of the corticospinal tract. With this type of 3D map, bilateral hemispheric asymmetries of streamtubes were shown for all 5 cats. These data suggest that there are white matter asymmetries associated with the corticospinal motor pathway in cats; however, these asymmetries do not appear to be sexually dimorphic. These methods validate our ROI model as marked, our imaging techniques, and our computer analysis allowing us to move forward with other studies testing effects on the central nervous system in pathological conditions, such as in feline immunodeficiency virus, as well as the changes in white matter that result in differences in diffusion tensor signals.

Student Support: NIH Grant #5T35RR007071

Genetic modification of the *Edwardsiella ictaluri dnaK*, *arnA*, and *typA* genes for live vaccine development. Lauren Phillips*, Jingjun Lu, Michelle Banes, Mark L. Lawrence, and Attila Karsi
Enteric Septicemia of Catfish (ESC) caused by the bacteria *Edwardsiella ictaluri* is one of the capital bacterial diseases plaguing the aquaculture industry in the United States. Production losses and treatment cost the catfish industry millions of dollars annually. Current treatment methods consist of either coating feed with Terramycin, Romet, Aquaflor antibiotics after clinical signs appear or by administering the only commercially available live attenuated vaccine on catfish fry. However, limited success has been achieved in treating this important disease. Of the available treatments and preventatives, live attenuated vaccines provide a high level of protection against ESC. *E. ictaluri* is resistant to catfish serum, and we have already determined differentially expressed proteins of *E. ictaluri* in serum killing process. Thus the purpose of this study is to eliminate the functions of dnaK, arnA, and typA genes of *E. ictaluri*, showing differential expression during serum resistance. In-frame deletions of these genes were accomplished by gene splicing by overlap extension (SOE). Mutated DNA fragments were cloned into pMEG375 suicide plasmid, and the plasmids with mutated genes were transferred into *E. ictaluri* by conjugal mating, which will result in elimination of the functional chromosomal genes of *E. ictaluri*. Attenuated mutant *E.*

ictaluri is expected to be used as cost efficient modified live vaccines that could conceivably reduce the *E. ictaluri* outbreaks and profitable loss experienced by catfish producers.

Student Support: NIH Grant #5T35RR007071

Effect of LDL on stallion sperm motility after cryopreservation. Susan Rodgers*, Heath King, and Richard Hopper

Cryopreservation of sperm has been used for many decades in assisted reproduction for a wide variety of species. Cryopreservation places several stressors on cells, the most detrimental include, ice crystal formation, osmotic changes, temperature shock and physical manipulation. Spermatozoa of different species tolerate the cryopreservation process differently. For example, stallions do not tolerate the freezing process as well as bulls or humans. Current methods to improve freezability include altering the freezing media, freezing process and thawing protocol. This project will explore altering the freezing media. Egg yolk improves post thaw quality of cells, and it has been suggested that the cryoprotectant properties of egg yolk are found in the low density fraction that is primarily composed of low density lipoprotein (LDL). LDL has been shown to improve post thaw quality of sperm in dogs, bulls, boars, and rams. Simply adding clarified egg yolk plasma has been shown to improve stallion freezing quality. The goal of this project was to determine if the addition of LDL to freezing media could improve the post-thaw quality of stallion semen compared to that of semen frozen with clarified egg yolk plasma. Two extenders were used: INRA 96 and a homemade Modified Kenney extender. Egg yolk was clarified by centrifugation to remove many large particles that make computer assisted sperm analysis (CASA) difficult. LDL was extracted from fresh chicken eggs by a combination of centrifugation and induced salt formation. Four stallions of 3 breeds ranging from 4-18 years of age were each collected 8 times for a total of 32 collections. Motility was assessed post thaw with CASA (Hamilton Thorne Biosciences, Inc, Beverly, USA). A slightly higher post thaw motility was observed in the extenders containing LDL. The difference (improvement) in motility however was stallion dependent.

Student Support: Mississippi State University College of Veterinary Medicine

Comparison of virulence factors between animal and human *Staphylococcus aureus* isolates. R. Smith*, J. Y. Park, F. W. Austin, and K. S. Seo

Staphylococcus aureus is the frequent cause of a wide range of diseases in both humans and animals. Some strains appear to be more adapted to a particular species of host. However, recent studies have shown that various strains of *S. aureus* have evolved to cause disease outside of their most common hosts. More information is needed for us to understand how such adaptations occur. The objective of this study is to make a genetic comparison of virulence factors between human and animal *S. aureus*isolates. Multiplex PCR was performed to analyze isolates from both sources for the presence of 39 genes for superantigens, hemolysins, cell adhesion molecules, and other virulence factors. The most common virulence factors in the animal isolates were *hld* and *hlgA*, both present in 100% of the isolates. In the human isolates, 100% contained *hld, hlgA, hla, sssp, fbpA,* and *clfA*. The genes for LukF, LukS*,* and Ear were not found in the animal isolates. The least common virulence factor for the human isolates was Ear, being found in only 5% of the isolates. The most common superantigens in both animal and human isolates were SEI (68.4% and 65%, respectively), SEG (63.2% and 65%), and SEIN (63.2% and 60%). The superantigens *seb, see,* and s*el* were absent from the animal isolates, and *see* was absent from the human isolates. Also, SCC*mec* type IV was present in 66.7% of the methicillin-resistant animal isolates. This is a common SCC*mec* type in human strains, and was present in 81.8% of the resistant human isolates. The similarities between these animal and human isolates suggest that exchange of genetic material may indeed occur between these sources.

Student Support: Merial Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine

The Bovine Spongiform Encephalopathy Phenotype Database. Tracy Thompson*, Shane Burgess, and Fiona McCarthy

Bovine Spongiform Encephalopathy (BSE) of cattle is a progressive neuropathy that affects beef and dairy industries worldwide and is a zoonosis. Furthermore, atypical forms of BSE have emerged making BSE diagnosis and control, as well as understanding its pathogenesis more challenging. Atypical BSE usually affects older cattle and has a different incubation period than classical BSE. Although there are currently more than 3,000 papers PubMed-indexed BSE papers, the knowledge in these papers is not readily accessible because: not all researchers and clinicians have access to all publications; the experimental details cannot be searched without reading the entire paper; and the time taken to do this reading is not economically sustainable . Moreover, because a normal consequence of human free text is term ambiguity and redundancy accurate computational text mining for the relevant and useful information is currently technically impossible. An online database allowing access BSE phenotype data would transform BSE knowledge recovery, and thus BSE diagnosis, control and understanding. As atypical BSE is an emerging issue we first identified the 58 papers containing experimental data for atypical BSE published (as of July 13, 2011). We used enumerated bio-ontologies for standardized experimental data collection and reporting as bio-ontologies provide computationally-amenable, as well as human readable, consistent term definitions and also facilitate data querying and sharing. We manually biocurated the atypical BSE experimental literature, and derived over 700 BSE phenotype annotations. This includes information about BSE type; host genotype, sex, age and tissue sampled; incubation period, clinical duration; clinical signs; and assays and assay reagents. Notably, these annotations include data from both BSE and atypical BSE and these annotations are linked to the original papers. We are currently making this data web accessible as the *Bovine Spongiform Encephalopathy Phenotype Database* as part of the AgBase databases (www.agbase.msstate.edu).

Student Support: Agriculture and Food Research Initiative Competitive Grant no. 2011-67015-30332 (USDA National Institute of Food and Agriculture) and the Mississippi State University College of Veterinary Medicine.

Age-related changes in dopamine neurochemistry and gene expression in dopamine neurons of the olfactory bulb In Nurr1-null heterozygous and wild-type mice. Samantha N. Vitale*, Shirley Guo-Ross, and Jeffrey B. Eells

The majority of the dopamine neurons found in the olfactory bulb are located in the glomerular layer. Dopamine modulates the processing of sensory information in the olfactory bulb by depressing neurotransmission when it binds to D2 receptors. The NR4A transcription factors (Nurr1 and Nur77) have been implicated in the regulation of olfactory function and dopamine transmission. Nurr1, especially, has been shown to regulate expression of dopamine neurotransmission genes in mesencephalic dopamine neurons. Previous data has shown that aging alters olfactory function. The purpose of the current project is to better understand how aging affects olfactory bulb function and the roles of NR4A transcription factors within the dopamine neurons of the olfactory bulb in possible regulation of dopamine neurotransmission genes. Using a Laser Capture Microdissection System, the glomerular layer of the olfactory bulb was isolated from either young (4 months) or aged (15-16 months) and either Nurr1-null heterozygous (Nurr1 +/-) or wildtype (Nurr1 +/+) mice (n=8-9/group). RNA was extracted from the captured dopamine neurons, and we used quantitative real time PCR to determine the expression of Nurr1, Nur77, tyrosine-hydroxylase (TH), and dopamine transporter (DAT). Additionally, dopamine and metabolite levels were determined in the olfactory bulb of these groups. Aging significantly elevated tissue dopamine levels similarly across genotypes. DOPAC levels, however, were significantly lower in the aged +/- mice. Usable RNA was isolated from the dissected tissue, as values for the internal control gene β-actin were obtained using quantitative PCR from 33 out of 34 samples. However, only 17 out of the 34 samples amplified using TH primers, and 21 out of 34 samples amplified using Nur77 primers. No consistent trend in amplification was found based on age or genotype. Although aging significantly increases tissue dopamine in the olfactory bulb, the molecular mechanisms responsible remain to be determined.

Student Support: NIH Grant #5T35RR007071