Front row (l to r): Julie Birch, Casey Graves, Lauren Belser, Holly Smith, Lisa Quinn, Katy Fogt
Second row: Sarah Morse, Emily Pearce, Rebecca Brezinsky, Jennifer Cain, Brittany Moore-Henderson
Third row: Bayard Grillis, Michael Diehl, Khalilah May, James Nichols, Dr. Andrea Varela-Stokes (mentor)
Back row: Dr. Jean Feugang, Dr. Mark Lawrence, Dr. Jeff Eells (mentors)
Not pictured: Blair Snively

***Ehrlichia chaffeensis* and intestinal parasites in Mississippi shelter dogs**

Lauren Belser*, Neely Alberson, Whitney Smith, Chelsea McIntosh, Linda Pote, Phil Bushby, Kimberly Woodruff, Andrea Varela-Stokes

Zoonotic diseases are becoming increasingly important due to the close relationship many people have with companion animals. *Ehrlichia chaffeensis* is the causative agent of human monocytic ehrlichiosis, a zoonotic tick-borne disease that can also affect dogs. The tick vector for *E. chaffeensis, Amblyomma americanum,* is readily found in Mississippi. The goal of this project was to evaluate shelter dogs in the Mississippi Delta, a historically unstudied area, for evidence of *E. chaffeensis* infection and exposure. In addition, dogs were evaluated for zoonotic and other gastrointestinal parasites. We collected whole blood and serum samples from 77 shelter dogs at 4 shelters in the Mississippi Delta. A nested PCR assay was used on DNA extractions of whole blood to detect circulating *E. chaffeensis*. An indirect fluorescent antibody test was used to detect antibodies in serum or plasma. Additionally, centrifugal floatation was performed on 39 fecal samples to identify gastrointestinal parasite ova. Thus far, 8 of 77 dogs evaluated were positive for *E. chaffeensis* DNA by PCR. Antibodies to *E. chaffeensis* were found in 21 of 72 canine samples. Zoonotic parasites identified in fecal samples included *Ancylostoma caninum* and *Toxocara canis.* Non-zoonotic parasites including *Isospora* spp., *Sarcocystis* spp. and *Trichuris vulpis* were also observed. Results demonstrate evidence of a zoonotic tick-borne agent in shelter dogs from an understudied region in Mississippi, suggesting humans may be at risk of *E. chaffeensis* infection here. The gastrointestinal parasites detected were not surprising considering the greater likelihood of exposure and lack of access to treatment in shelter dogs.

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

Virulence and efficacy of an *E. ictaluri* mutant in catfish fry
Julie Birch*, Safak Kalindamar, Michelle Banes, Hasan Tekedar, Mark Lawrence, Attila Karsi

Edwardsiella ictaluri is the causative agent of Enteric Septicemia of Catfish (ESC), a disease that is devastating to the U.S. aquaculture industry. Of the possible treatments and preventatives, live attenuated vaccines have the potential to provide a high level of protection against ESC. However, there is significant need for novel and more effective vaccines. The purpose of this study was to determine the vaccine potential of a live attenuated strain of *E. ictaluri,* designated EiX. To assess the efficacy and optimal protective dose of EiX, catfish fry were vaccinated with EiX by immersion at concentrations of 10^9 - 10^5 colony forming units (CFU/ml of tank water) and challenged with a lethal dose (10^7 CFU/ml) of wild type *E. ictaluri* 21 days later. Vaccination challenges showed that EiX is completely attenuated at 10^8 CFU/ml and caused only X% mortality at the highest dose of 10^9 CFU/ml. The best protection was obtained at the dose of 10^8 where we observed X% mortality after challenge with wild type *E. ictaluri*. Although the vaccine vas very attenuated at 10^8 CFU/ml and lower, protection potential declined with lower vaccination doses. Results indicate that EiX is a highly attenuated mutant providing excellent protection for catfish fry. EiX seems to have a very good potential to prevent *E. ictaluri* infections in commercial catfish aquaculture.

Student Support: NIH 5T35OD010432

Expression of Aquaporins and Glucose Transporters in Cryopreserved Boar Spermatozoa
Rebecca M Brezinsky*, Antonio CM Filho, Ramey C Youngblood, Christy Steadman, Scott T Willard, Peter L Ryan, and Jean M Feugang

Sperm cryopreservation is a useful tool in agriculture for long-term storage, transportation, disease screening, and gene banking. While cryopreservation is used in livestock and companion animals, its application in swine is still limited due to the existence of “good-freezer” and “bad-freezer” boars. Cryopreservation is a traumatizing process to cells and its successful application requires the presence of proteins such as aquaporins (AQP) and glucose transporters (GLUT) for water and cryoprotectant influxes across the plasma membrane. The presence of these proteins in boar spermatozoa is not well-described, thus our study aimed at profiling their expression in these cells. Motile spermatozoa were purified from pooled semen of known “good-freezer” and “bad-freezer” boars for RNA isolation (n=3). Samples were quantified and pure RNA were used for cDNA synthesis, followed by semi and quantitative PCR amplifications using aquaporin (1-11) and glucose transporter (GLUT-3 and GLUT-5) primer pairs. Semi-quantitative PCR revealed differential expression of AQP isoforms, known as water (AQP-1, -5, and -11) and glycerol (AQP -3, -7, -9, and -10) transporters. Expression of AQP-9 and GLUT-3 transcripts were low, while AQP-2, -4, -6, and -8 as well as GLUT-5 were not detected. Quantitative PCR on selected genes showed a lower expression of AQP-10 in “good freezer” boar spermatozoa compared to “bad freezer” ($P<0.05$), and AQP-11 was unchanged ($P>0.05$). This study is the first step toward a comprehensive analysis of transporter proteins in boar spermatozoa. Their identification as potential players during cryopreservation may help improve sperm freezability that is currently a limiting factor in swine.

Student Support: Merial Veterinary Scholars Program, and Mississippi State University College of Veterinary Medicine
Research Support: USDA-ARS Biophotonics Initiative #58-6402-3-0120

Pre-lab factors affecting Tritrichomonas foetus culture and PCR test performance Jennifer M. Cain*, Carla L. Huston, Frank W. Austin, Terry Doler, Alejandro Banda, Lanny W. Pace, Kevin Walters, R. Hartford Bailey, David R. Smith

Tritrichomonas foetus is a venereally transmitted protozoal reproductive pathogen of cattle. Current diagnostic methods cannot prevent introducing subclinically-infected bulls into non-infected herds. The objective of these studies was to evaluate factors affecting T. foetus diagnostic test performance. Study 1: 600 selective enrichment pouches (InPouch TF®) were inoculated with bacteria from the prepuce of four healthy bulls and 30 T. foetus were inoculated into 300 pouches by random assignment. Pouches were randomly assigned to 20C pre-incubation treatments (0, 2 and 4 days). After holding, samples were incubated 37C 5 days. Cultures were examined microscopically (100x) days 1, 3, and 5 by readers blinded to treatment. One T. foetus inoculated sample was culture positive. PCR analysis is in progress. Study 2: 40 pouches were randomly assigned to 4 levels of T. foetus inoculation (67, 670, 6,700, or 67,000 organisms/pouch) and 2 levels of bacteria (inoculated or not with preputial bacteria passaged twice in selective enrichment media). Pouches were incubated 37C and examined microscopically (100 power) daily for 7 days by blinded readers. Sensitivity was affected by bacteria ($p<0.0001$). All pouches inoculated with bacteria were culture negative; however, T. foetus was detected at least once from all pouches without bacteria. Sensitivity increased daily in pouches without bacteria (OR= 4.5, $p = 0.02$), but did not differ by reader ($p = 0.39$). Study 3: 4 10-fold dilutions of

T. foetus were incubated 37C for 7 days. Lower inoculums peaked in concentration later. Number of organisms, bacterial contamination, and day post-inoculation may affect test sensitivity.

Student Support: Boyd and Anne Epperson Summer Research Award for Food Animal Medicine
Research Support: Hall-Davis Beef Cattle Health Fund and Mississippi State University College of Veterinary Medicine

Use of pMAD Shuttle Vector For Generating Gene Deletions in *S. pneumonia*

Michael Diehl*, Joo Youn Park, Bindu Nanduri, Keun Seok Seo

Streptococcus pneumoniae (pneumococcus), an encapsulated Gram-positive pathogen that typically colonizes the human nasopharynx, often extends beyond the surrounding nasopharyngeal tissues to cause bacterial pneumonia, otitis media, and meningitis. *S. pneumoniae* represents a serious public health concern, as it causes significant disease worldwide, particularly in developing nations. Previous studies identified the use of bacterial polyamines, which are small cationic compounds used in bacterial metabolism, as key players in the pathogenesis of pneumococcus. A specific ATP-binding cassette transporter system, the polyamine transport (Pot) operon, is involved in polyamine uptake and biosynthesis within pneumococcus. Previously, a novel strategy for the creation of gene inactivation mutants has been described in Gram-positive bacteria. The use of the temperature-sensitive shuttle vector, designated as “pMAD,” has been able to efficiently construct mutants in naturally transformable Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*, and is an innovative tool for developing gene replacement in bacteria and in the screening of recombinant mutants. The goal of this study is to establish a gene inactivation system in pneumococcus, using the pMAD shuttle vector, and apply it to inactivate Pot operons. Specifically, we are targeting PotD, which facilitates uptake of spermidine and putrescine, and PotA, which supplies energy in the process of polyamine uptake. We expect that the evaluation of the pMAD shuttle vector to inactivate these genes within *S. pneumoniae* will lead to a better understanding of these transporter proteins, the role of polyamines in bacterial pathogenesis, and yield important information regarding disease prevention in pneumococcal infections.

Student Support: NIH 5T35OD010432

Thorough analysis of virulence factors and MGE markers in *Staph. aureus,* isolated from bovine mastitis

Katy Fogt*, Hyang Mi Nam, Bo Youn Moon, Joo Youn Park, Suk Kyung Lim, Suk Chan Jung, Yong Ho Park, Ye Ji Lee, Keun Seok Seo

Staphylococcus aureus is an important opportunistic, zoonotic pathogen that causes a wide range of diseases in humans and animals. The major challenge in understanding the pathogenesis of *S. aureus* infections is largely due to the specific and redundant activities of a battery of its virulence factors. Whole genome sequencing and other analysis have shown that livestock associated-methicillin resistant *S. aureus* (LA-MRSA) possess common virulence factors as human-MRSA but have also unique virulence factors that might be important to adapt in specific hosts. Importantly, most virulence factors are associated with mobile genetic elements such as prophages, staphylococcal pathogenesis islands, chromosomal cassettes, and plasmids, allowing a horizontal transfer of virulence factors to other *S. aureus* that may expand the host range. In this study, we investigated the presence of 56 major virulence factors (cell attachment factors, cytotoxins, and superantigens) and MGE markers in *S. aureus* strains (n=207) isolated from bovine mastitis using multiplex PCR. Results showed that several cell attachment factors such as Bsa, Ebh, and Emp were highly conserved (88.4 – 97.6%), suggesting these factors may contribute *S. aureus* adaptation to bovine. Similar to human-MRSA, enterotoxin gene cluster were the most prevalent superantigen genes (30.4%). Cytotoxin genes encoding LukD/E, and HlgA/B/C were highly observed (89.9 – 91.3%). Importantly, the genes encoding Bsa, enterotoxin gene cluster, and LukD/E are associated with the chromosomal cassette, υSaβ, and the prevalence of υSaβ in *S. aureus* isolated from various geographical settings has rapidly increased in last decade, suggesting the horizontal transfer of this elements between strains.

Student Support: Boyd and Anne Epperson Summer Research Award for Food Animal Medicine

Developmental Pesticide Effects on the Endocannabinoid System in Juvenile Rat Brain
Casey A. Graves* and Russell L. Carr

There is concern that the organophosphate insecticide chlorpyrifos (CPS) is causing toxicity in children following low level developmental exposure. It has been hypothesized that these effects occur through a mechanism other than its traditional cholinergic target, cholinesterase (ChE). We have recently identified that the endocannabinoid system, a system vital to proper nervous system development, may be a target. Repeated developmental exposure to CPS results in greater inhibition of the fatty acid amide hydrolase (FAAH), the enzyme that metabolizes the endocannabinoid anandamide (AEA), than inhibition of either ChE or monoacylglycerol lipase (MAGL), the enzyme that metabolizes the endocannabinoid 2-arachidonylglycerol (2-AG). This inhibition of FAAH leads to accumulation of AEA in the juvenile rat brain. To determine if this inhibition and accumulation occurs following exposure levels that do not affect the cholinergic system, 10 day old rat pups were exposed daily for 7 days to either corn oil or 0.5 mg/kg CPS by oral gavage. At 4 and 12 hrs post- exposure, serum ChE and carboxylesterase activities were inhibited. However, forebrain ChE was not inhibited suggesting no effects on the brain cholinergic system. There was no inhibition of MAGL or accumulation of 2-AG but there was significant inhibition of FAAH resulting in marked accumulation of AEA in the forebrain. Although these data suggest a non-cholinergic target for CPS and a potential mechanism for toxicity, it is not clear if impacting the endocannabinoid system plays a role in the developmental toxicity of CPS.

Student Support: NIH 5T35OD010432

***Edwardsiella ictaluri* in Zebrafish: Bioluminescence and Fluorescence *in situ* Hybridization**
Bayard Grillis*, Wes Baumgartner, Claudia Hohn, Lora Petrie-Hanson, Mark Lawrence

Edwardsiella ictaluri is the causative agent of enteric septicemia of catfish (ESC), an economically important disease of channel catfish, which is the largest aquaculture species in the U.S. Recently, zebrafish have been established as a model for studying ESC, which suggests that *E.ictaluri* could also be a potential pathogen of zebrafish in research facilities. However, the pathogenesis of *E. ictaluri* in zebrafish and catfish remains poorly characterized. Therefore, the purpose of this study was to elucidate the site(s) of entry and progression of *E. ictaluri* infection in adult zebrafish by employing novel techniques, such as bioluminescence imaging (BLI) and fluorescence *in situ* hybridization (FISH). Adult zebrafish were exposed to *E. ictaluri* (93-146) pAK*gfplux1* via different routes of exposure: gastric gavage, bath immersion, and intraperitoneal injection. At 0, 0.5, 6, 24, 48, 72, 96, 120, and 144 hours post-infection, zebrafish were anesthetized, imaged for bioluminescence, sacrificed, and processed for FISH. BLI revealed bacterial signal originating from unique locations for gavage (intestines, gills), immersion (nasal bulbs, integument), and injection (abdominal viscera) treatments, respectively. BLI showed increasing signal in injected fish up to 72 hours post-infection; at this point, almost all injected fish succumbed to infection. In contrast, gavage and immersion treatments initially showed increasing signal through 72 and 24 hours, respectively, followed by decreasing signal through 144 hours. FISH enabled pathogen detection for gavage (intestinal mucosa/submucosa, 24 hours), immersion (nasal bulbs, 24 and 48 hours), and injection (hematopoietic organs, 48 hrs) treatments, respectively, which correlated well with BLI data. In conclusion, these results indicate that: 1.) *E. ictaluri* possesses multiple routes of entry in the zebrafish model; 2.) BLI data is accurate in localizing infection in zebrafish; and 3.) FISH is a simple and effective technique for visualizing *E. ictaluri* in histology sections.

Student Support: NIH 5T35OD010432

Effect of WIN55.212-2 on canine endothelial cells proliferation
Khalilah May*, Sandra C. Bulla, Matthew Ross, Kari Lunsford, Camilo Bulla.

Tumor angiogenesis is regulated by pro- and anti-angiogenic factors produced by tumor cells and stroma. Thus, inhibition of tumor angiogenesis can be achieved by decreasing pro-angiogenic or increasing anti-angiogenic factor production, or directly inhibiting endothelial cell proliferation. WIN-55,212-2 is a synthetic cannabinoid that has been demonstrated to inhibit vascular endothelial growth factor (VEGF) expression in human, mouse and recently, in canine osteosarcoma cells, making it a potential cancer treatment through angiogenesis inhibition. The aim of this study was to determine the effect of WIN-55,212-2 on canine endothelial cells (CEC). CECs were obtained from abdominal aorta, cultured under standard parameters and incubated with VEGF enriched media. CECs were then incubated with 1µM WIN-55 for 48 hours. RNA was extracted and expression of RCAN-1, HBEGF, PTGS2, and MMP2 genes were analyzed by comparative Cq method. Statistical analysis demonstrated no difference in expression in any of the genes tested between the treatments,

indicating that, apparently, there was no inhibition or stimulation of endothelial cell proliferation by WIN-55,212-2. These findings suggest that WIN-55,212-2 does not interfere with canine endothelial cell proliferation thus further indicating that tumor treatment with WIN-55,212-2 may cause inhibition of angiogenesis by inhibiting VEGF-A expression by tumor cells.

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

Potential Hosts for *Amblyomma maculatum* Ticks and Transmitted Pathogens

Brittany Moore-Henderson*, Jung Keun Lee, Whitney Smith, Chelsea McIntosh, Gail Moraru, Andrea Varela-Stokes

The Gulf Coast tick, *Amblyomma maculatum*, is a known vector for the canine protozoal pathogen, *Hepatozoon americanum,* and the emerging human pathogen *Rickettsia parkeri.* Identifying vertebrate reservoirs for *A. maculatum*-transmitted pathogens is a significant step towards understanding natural maintenance of these pathogens. In order to identify potential reservoirs, we used reverse line blot hybridization (RLBH). RLBH has been used to identify bacterial strains and was recently adapted to identify vertebrate hosts previously fed on by hematophagous arthropods. In this study, we used RLBH to evaluate 61 unfed adult *A. maculatum* for host DNA in remnant blood meal. We amplified a portion of vertebrate mitochondrial 12S rRNA gene and hybridized products to selected vertebrate oligonucleotides including rodent, artiodactyl, canid, felid, bird, lizard, and human. We concurrently tested tick extracts for *H. americanum* and *R. parkeri,* using PCR to amplify portions of the 18S rRNA and *rompA* genes, respectively. Through this approach, we identified hosts including rodents, birds, canids, and artiodactyls for which remnant DNA was detectable. In addition, 21/77 ticks were positive for *H. americanum* and 14/ 77 were positive for *R. parkeri* by PCR; results were confirmed for *R. parkeri* but not *H. americanum*. Overall, avian and rodent DNA was detected most often in ticks, including ticks positive for *R. parkeri,* in which 44% (4/9) fed on avian hosts. These findings support rodents and birds as important hosts for *A. maculatum* and suggest they play a role in natural maintenance of *A. maculatum*-transmitted pathogens.

Student Support: NIH 5T35OD010432

Interactions between novel probiotic and pathogenic *E. coli*

Sarah Morse*, Jessica Grissett Wilson, and Janet R. Donaldson

Probiotics are microorganisms that are commonly used both in human medicine and commercial farming to increase resistance to disease through alteration of the gut microflora. Recently, our research group has identified a potential probiotic (*Enterobacter sp*) that has a unique capability of increasing the availability of energy to the host. In order to further investigate the potential use of this bacterium, two aspects related to probiotic mechanisms of action were examined *in vitro*. Viability of cultures combining both *Enterobacter* and *Escherichia coli* were examined to determine whether nutrient availability, and therefore growth, would differ for *E. coli* in the presence of the potential probiotic. The five serotypes of Shiga toxin-producing *E. coli* used in this study, O157:H7, O103:H8, O111:H8, O145:H28, and O26:H11, were tested in combination with *Enterobacter* sp. Viability of *Enterobacter* did not significantly change in the presence of any of the five serotpyes of *E. coli* tested. *Enterobacter* was also found to have a strong binding affinity to colonic epithelial cells GPC16. Additionally, *Enterobacter* may impede binding of *E. coli* to colonic epithelial cells, though further research is needed to confirm this observation. Together, these data suggest that the novel *Enterobacter sp.* may limit the activity of *E. coli in vivo*. This could potentially impact the prevalence of *E. coli* in livestock, which could in turn reduce potential for contamination of food products.

Student Support: NIH 5T35OD010432

Tendons from Horses with HERDA have Abnormal Elastic Modulus

JM Nichols*, JE Bowser, SS Patnaik, S Kumar T K, J Liao, CE Swiderski

Hereditary Equine Regional Dermal Asthenia (HERDA) is an autosomal recessive collagen disorder of Quarter Horses caused by a c.115G>A mutation in the gene encoding Cyclophilin B. HERDA heterozygotes are over-represented among elite performers in the western performance discipline of cutting, leading to concerns that HERDA carriers possess physical attributes that confer a performance advantage. Congruent with the role of Cyclophilin B in post-translational collagen modification, we previously demonstrated significant reduction in tensile strength of tenoligamentous tissue from horses homozygous for the HERDA allele (HERDA horses) relative to horses without the HERDA allele (control horses). Differences in elastic modulus, or stiffness, correlate to altered biomechanical efficiency of work in tendons leading to the hypothesis that the stiffness of tendon is modified by the HERDA mutation − an outcome that could modify the

biomechanical efficiency of HERDA tendon. Using a previously validated sensitive biaxial testing device, the stiffness of the superficial (SDFT) and deep digital flexor tendons (DDFT) from HERDA horses and control horses were quantified in orientations that were both perpendicular and parallel to tissue collagen fibers. Tissues were assayed in triplicate from 3 HERDA horses and 3 age-matched controls. With distraction forces oriented perpendicular to the collagen fibers, SDFT and DDFT from HERDA horses were stiffer than control samples. This difference was most prominent for DDFT. With distraction forces parallel to tissue collagen fibers, a trend to reduced stiffness was evident in DDFT but not SDFT from HERDA horses. These results confirm the HERDA allele modifies the stiffness of tendons in HERDA homozygotes and that the magnitude of these differences varies across different tendons. Further, when comparing the biomechanical properties of tissues to assess the role of the HERDA allele, the orientation of distractive force relative to the tissue's collagen fiber orientation should be standardized for accurate comparisons.

Student Support: NIH 5T35OD010432

Use of Thermal Imaging for the Measurement of Body Temperature in Animal Species

Emily Pearce* and Scott Willard

Veterinarians monitor body temperature to evaluate the health of animals; however, this can be difficult with non-domestic species. Digital infrared thermal imaging (DITI) permits the noninvasive monitoring of surface temperatures, and has been used to correlate body temperature to external measures (e.g., eye, muzzle, skin/coat). The objective of this study was to explore the use of urine temperature as a correlate to core body temperature. Holstein (n=82) and Jersey (n=29) lactating dairy cows were imaged, multiple times, using DITI (FLIR SC660 camera) on multiple days (8 days; 11 sessions: am/pm). 1,232 DITI images were acquired for the following parameters: eye, muzzle, skin/coat and urine stream; the maximum temperature was used for analysis. Rectal temperatures were used as the standard for comparison, and correlations and mean comparisons were calculated. Urine temperature was moderately to highly correlated ($P<0.05$) with rectal temperature ($R=0.43$ to 0.75). Rectal temperature was correlated ($P<0.05$) with other temperature measures at a lower level (R=eye, 0.50; muzzle, 0.39; skin, 0.28). There were noted differences among the cameras used, time of day (am/pm) and environmental conditions (ambient temperature/relative humidity). Preliminary analysis of DITI temperatures from urine imaged mid-stream vs. urine collected in a cup demonstrated that urine imaged mid-stream was more similar to rectal temperature (0.58℃ vs. 1.48℃ difference from rectal, respectively). These data suggest that DITI is a viable method of monitoring urine temperature as a correlate to body temperature when imaged mid-stream in cattle, and may have value for applications in both domestic and non-domestic species.

Student Support: Merial Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine
Research Support: Provided, in part, by the USDA-ARS funded "Biophotonics Initiative"

Antigenic characterization of H3N2 seasonal influenza viruses from Minnesota, 2012-2013

Lisa Quinn*, Elizabeth Bailey, Hailiang Sun, Zhixin Feng, Brigitte Martin, Sara Vetter, and Xiu-Feng Wan

The seasonal A virus H3N2 causes influenza epidemics in people across the world every year. Antigenic change caused by mutations of H3N2 allows this virus to evade herd immunity acquired from previous infections and vaccinations. Previous studies suggested that some areas seemed to have a higher number of infections than others. Here we hypothesize that antigenic drift of influenza A viruses would occur more frequently in some geographic locations than others. In this study, we tested this hypothesis by antigenic analyses of a collection of H3N2 isolates from Minnesota, 2012-2013 season (n=179, 175 known and 4 unknown zip codes). A set of ferret antisera were generated by nasally infecting A/Brisbane/10/2007(H3N2), A/Perth/16/2009(H3N2), A/Victoria/361/2011(H3N2), A/Minnesota/300500/2013(H3N2), A/Minnesota/307592/2012(H3N2), or A/Mississippi/17/2013(H3N2). The serological method hemagglutination inhibition (HI) assays are then used to determine the amount of antibodies needed to prevent the virus from agglutinating turkey red blood cells. The HI titers were used to differentiate antigenic properties and to identify antigenic variants among those H3N2 isolates from Minnesota. We have finished the HI results with A/Brisbane/10/2007(H3N2), A/Perth/16/2009(H3N2), and A/Victoria/361/2011(H3N2). Our results demonstrated that these H3N2 isolates had highest across activities against A/Victoria/361/2011(H3N2), followed by A/Perth/16/2009(H3N2) and A/Brisbane/10/2007(H3N2). Preliminary analyses showed that approximately 50 out of 180 had at least a 4log2 fold change in HI value against A/Brisbane/10/2007(H3N2) versus A/Victoria/361/2011(H3N2). These antigenic variants are located across 38 areas with unique zip codes, and 27 of these isolates are geospatially located in Minneapolis-St. Paul areas. More complete antigenic characterization using antigenic cartography and geospatial analyses will be carried out further by including the anti-sera of A/Minnesota/300500/2013(H3N2), A/Minnesota/307592/2012(H3N2), and A/Mississippi/17/2013(H3N2) towards validating our hypothesis.

Student Support: NIH 5T35OD010432

Effects of *T. gondii* on Nurr1-Null Heterozygous Mice as a Model of Schizophrenia
Holly M. Smith*, Jeffrey B. Eells, and Andrea Varela-Stokes

Toxoplasma gondii is a highly successful parasite, which can infect virtually all warm-blooded animals. It is best known for its ability to alter behavior in rodents resulting in a more efficient transmission to its only definitive host, felids. In the U.S., it is estimated that 22.5% of the population 12 years and older have been infected with *Toxoplasma*. Recent research has found an association between *T. gondii* infection and schizophrenia based on antibody titers to the parasite and alterations in dopamine neurotransmission. In our study, we used Nurr1-null heterozygous mice, which have alterations in dopamine neurotransmission and altered dopamine neurotransmission related behaviors. Our current hypothesis is that *T. gondii* infection will have a greater effect on dopamine related-behavior in Nurr1-null heterozygous mice and that these changes are due to a difference in immune/cytokine response. To test this hypothesis, we measured the activity level, fear/anxiety level, startle response, prepulse inhibition, and approach/avoidance to novelties and/or bobcat urine in Nurr1-null heterozygous mice and wild-type littermates prior to and after infection with *T. gondii* and in uninfected controls. Serum antibody titers demonstrated that mice had been infected, although most mice had high titers, some infected mice had low and negative titers. Additionally, conditions for induction of cytokine expression from peritoneal macrophages have been established. Preliminary results suggest that the Nurr1-null heterozygous mice have elevated open-field activity and are more vulnerable to the behavioral changes associated with *T. gondii* infection. In the future, we will use the antibody titers as a covariant with the behavioral changes. Additionally, we will determine cytokine induction in peritoneal macrophages from Nurr1-null heterozygous mice to test the hypothesis that the host immune response contributes to the alteration of behavior.

Student Support: NIH 5T35OD010432

The thermal effects of protective sports boots on the equine forelimb
Blair C. Snively*, Jacquelyn E. Bowser, Ben Nabors, Robert L. Linford

The use of protective boots on sport horses has risen over the last 20 years; however, tendon injuries, especially those involving the superficial digital flexor tendon (SDFT), are still a common occurrence during equine competitions. Temperatures between 42 and 43°C cause fibroblast cell damage and death; tendon hyperthermia is one of several proposed causes of tendon degeneration. The SDFT of the bare unbooted forelimb of the horse at a gallop can reach core temperatures of up to 45°C and adding a boot could easily trap heat against the leg and dangerously increase flexor tendon temperatures. This study was undertaken to fill an unmet need to investigate if temperatures within sports boots are reaching dangerous levels during exercise. We monitored 12 polo ponies, both booted and unbooted, at rest and after strenuous exercise. The mean ± SD pre-exercise temperatures were 28.51 ± 4.11°C and 26.93 ± 4.01°C respectively, in the booted and unbooted leg, whereas, the post-exercise temperatures were 40.26 ± 1.95°C and 33.15 ± 1.24°C, respectively, in the booted and unbooted legs. Temperature in the booted leg increased 11.75 ± 3.42°C with exercise ($p<0.0001$) and was 7.11 ± 1.90°C higher than the post-exercise temperature in the unbooted leg ($p<0.0001$). Intense polo training exercise caused a 6.22 ± 4.14°C increase in limb temperature and adding the boot caused an additional 5.53 ± 2.25°C rise in temperature with exercise. The study revealed that the boot causes a significant increase in limb temperature and that the effect is exacerbated by exercise.

Student Support: Morris Animal Foundation
Horses and logistical support provided by Charles Muldoon, Summer Hill Farm, Poolesville, MD.

Participating at Other Institutions

Investigations into the Polygenicity of an Autoimmune Lymphoproliferative Disease
Martha Frances Dalton*, Bernice Lo, Koneti Rao, and Michael Lenardo.

Many genetic disorders are proving to be polygenic in nature as opposed to resulting from a single mutation in a “disease gene.” This series of experiments is aimed to provide insight into the role of polygenicity in complex disease phenotypes. A father and son have an autoimmune lymphoproliferative disease, with their most remarkable clinical observation being persistent infiltration of T lymphocytes into non-lymphoid organs, including lung and brain. Mutations were found in two genes that play important roles in inflammatory responses: Gene1 is involved with tumor necrosis factor (TNF) production, and Gene2 plays a role in TNF-induced apoptosis of inflammatory cells. The mutations present in Gene1 and Gene2 could be collaborating to cause the disease phenotype of lymphoproliferation and infiltration: the Gene 1 mutation favoring

persistent inflammation, and the Gene2 mutation resulting in the impaired ability to terminate an immune response. Mutagenesis techniques were explored to determine how best to replicate the same two mutations present in the patients. We intend to transduce the mutated genes via lentiviral vectors into murine bone marrow derived monocytes to test for a similar cellular phenotype as seen in the patients: overproduction of TNF and a potential deficiency in TNF induced apoptosis. A technique of harvesting bone marrow derived cells and culturing them with macrophage colony-stimulating factor (M-CSF) was explored to maximize monocyte yield. Monocytes were selected because they can both produce and respond to TNF. We intend to create an animal model for the disease by nonsurgically instilling the double-mutant expressing monocytes into mouse airways to test for persistent inflammation and recruitment of other inflammatory cells to the lungs. This project will be the first to demonstrate how the combined effect of mutations in two different genes could synergistically result in a complex autoimmune disease.

Student Support: Comparative Biomedical Scientist Training Program, NCI
Research Support: NIH Intramural Research Program, National Institute of Allergy and Infectious Diseases

Characterization of a novel small regulatory RNA Bsr7 from *Brucella abortus* 2308

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Brucella, a member of the alphaprotobacteria family, are coccoid, Gram-negative, facultative intracellular pathogens. One of the most important zoonotic pathogens worldwide, *Brucella* is also the causative agent of human brucellosis. *Brucella* virulence is linked to several factors, one of which is the protein Hfq. This protein is an RNA chaperone whose primary function involves post-transcriptional gene regulation by mediating interactions between small RNAs and messenger RNAs. Small regulatory RNAs (sRNAs) are essential for bacterial gene regulation and range in size from 50-300 nt. RNA sequencing was performed in our laboratory to identify sRNAs, and to date, we have identified 14 novel sRNAs in *B. abortus* 2308. In this study we worked with the small regulatory RNAs Bsr7 and Bsr4. Northern blot analyses were performed on sRNAs Bsr7 and Bsr4 to identify expression levels under biologically relevant conditions, such as oxidative stress, nutrient deprivation, low pH, and iron levels. We determined that Bsr7 is responsive to iron as it was more highly expressed in iron replete conditions compared to low iron conditions. This suggests that Bsr7 may play a role in iron homeostasis of *B. abortus* 2308. We also generated a *bsr7* mutant to elucidate the biological role of this sRNA in *Brucella*. The *B. abortus* 2308 *bsr7* mutant will be used in future studies, such as macrophage infection assays, a mouse model of chronic *Brucella* infection, iron homeostasis analysis, and microarray analysis. In characterizing Bsr7 we discovered a potential link to iron homeostasis, and the *bsr7* mutant strain will allow us to explore its significance in *Brucella abortus* 2308.

Student Support: Merial