Student Abstracts 2010

First row (L to R): Sheila McShane, Kelly McDaniel, Aimee Daniel, Cassie Bayer, Rebecca Flores, Sarah Tankersley, Courtney Bowers
Second row (L to R): Michael Harlow, Dustin Russell, Brolin Evans, Zachary Brown, Joshua Barker, Ryan Black, Cynthia Miller, Diana Link
Third row (L to R): faculty mentors Dr. Jean Feugang, Dr. David Christiansen, Dr. Andrea Varela-Stokes, Dr. Jeff Eells, Dr. Mark Lawrence, and Dr. Linda Pote.
Not pictured: Amy Almo, Brett Weathers

Impact of Positive and Negative Predictive Values on Interpretation of Diagnostic Tests. A. J. Almo* and R. W. Wills

In the majority of companion animal clinics, the preferred method of diagnostic testing for many diseases is through serological test kits. When interpreting results from these tests, a clinician should be first aware of the positive and negative predictive values of these tests and their importance. The purpose of this research was to illustrate the impact of positive and negative predictive values in interpreting small animal diagnostic tests frequently used in small animal hospitals across the United States. The two serological test kit manufacturers that were assessed were Idexx, Inc. and Synbiotics. The specific diseases examined were as follows: lyme disease, heartworm, feline leukemia. The mean sensitivity and specificity were obtained directly from the manufacturer and from the scientific literature. The prevalence values of each disease tested were taken from the scientific literature as well. Curves of positive and negative predictive values were created for the different tests by plotting against a set of prevalence values. As the prevalence increased, the positive predictive value increased. Likewise a decrease in prevalence caused an increase in negative predictive value. The impact of

this was shown by finding the positive and negative predictive values for specific regional prevalences. In order to further illustrate the impact of positive and negative predictive values, a binomial distribution was simulated using the standard deviations of sensitivity, specificity, and prevalence. These results showed the various values that can be obtained for positive and negative predictive values. These findings demonstrate the importance of prevalence along with sensitivity and specificity of a test and positive and negative predictive value when interpreting diagnostic test results.
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Development of a Novel Real-Time Paradigm For Monitoring *E. coli* O157:H7: Photonic Growth Patterns of Bioluminescent *E. coli* O157:H7 Transformed With Two Unique Plasmids. J.S. Barker*, P. R. Broadway, J. Behrends, K. Moulton, J. O. Buntyn, S. Willard, P. Ryan, and T. B. Schmidt
Understanding the photonic growth properties of transformed *E. coli* O157:H7 for use in biophotonic *in vivo* monitoring and pathogen reduction is vital to the development of a real time paradigm. This trial's objective was to evaluate the photonic growth pattern of *E. coli* O157:H7 transformed with two unique photonic plasmids (XEN-14 transposon and pAK-1 lux). Both plasmids have antimicrobial-resistant genes inserted to provide selective pressure: XEN-14 for kanymycin (KAN) and pAK-1-lux for ampicillin (AMP). For both plasmids, 1 ml of overnight culture was placed into four flasks. For the XEN-14 plasmid, 2 flasks contained Tryptic Soy (TS) broth and 2 flasks contained TS+KAN broth. For pAK1-lux plasmid, 2 flasks contained TS broth and 2 contained TS+AMP broth. Cultures were incubated at 37oC for 14 hrs, during which aliquots were sampled and either analyzed for OD (600nm) or placed in a black 96-well plate and imaged. After imaging, each well was serially diluted in corresponding broth flask agar. Plates were incubated for 12 hours, then total CFU, emitting CFU and relative light unit (RLU) per sec determined. For XEN-14, there was no significant difference between cultures. For pAK1-lux, total CFU and emitting CFU for TS were significantly increased compared to TS+AMP (8.78 and 8.12 log CFU/ml, respectively) for hours 3-14, while there was no significant difference in RLU/s. These data characterize the photonic growth pattern for *E. coli* O157:H7 transformed with the XEN-14 and pAK1-lux, a vital step in the development of a novel-real time model for monitoring *E. coli*O157:H7.

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

Effects of ICPF in murine *Salmonella* peritonitis models. C. Bayer*, C. Matyi, K. Willeford, and B. Willeford
Immune Cell Potentiating Factor (ICPF) is a naturally occurring peptide isolated from serum which promotes a non-specific boost of the immune system, presumably by mediating production of secondary messengers. ICPF has been shown to decrease mortality in a Swiss Webster murine Salmonella peritonitis model. Toll-Like Receptor-4 (TLR4) is regarded as a major component of the immune cascade for gram negative infections. If ICPF works in association with TLR4, mice deficient in TLR4 would be expected to show increased morbidity and mortality in a Salmonella peritonitis model regardless of ICPF administration. The models presented here use a spontaneous knockout strain of mice deficient in TLR4 (C3H/HeJ), as well as a genetically compatible TLR4 positive sister strain (C3HeB/FeJ), and a strain previously shown to be positively affected by ICPF (Crl:CFW). Studies performed with these models include survivability and assessment of bacterial proliferation via fluorescent IVIS imaging and colony forming units (cfu) derived from harvested splenic tissue. On day -1, mice were injected intraperitoneally (IP) with 5 mg of either ICPF (+) or Phosphate Buffered Saline (dPBS) (-). On day 0 all mice were injected IP with a lethal dose of Salmonella. All groups of mice treated with ICPF experienced reductions in bacterial proliferation which resulted in higher survival [Percent Survival: C3H/HeJ TLR4-/- showed 87 vs. 7, C3HeB/FeJ showed 100 vs. 13, and Crl:CFW showed 73 vs. 7]. Collectively, these results show that ICPF does not have to work in concert with the TLR4 receptor to function.

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Comparative follicular fluid proteome analysis of healthy, developing porcine ovarian follicles. R. M. Black*, J. M. Feugang, Z. A. Brown, K. Pendarvis, S. Willard, and P. Ryan

The success of in vitro embryo production and related biotechnologies such as embryo transfer and cloning relies on the utilization of high quality oocytes. There is no reliable method for the selection of such oocytes within the follicle, and the identification and characterization of follicular fluid components may contribute to developing potential markers for selecting oocytes. Here, we performed a comparative proteome analysis of follicular fluids of developing porcine ovarian follicles. Sow ovaries were collected at a local abattoir followed by the dissection of follicles on ice. Follicles were grouped as medium (MF:3-6mm) and large (LF; 7-12 mm), according to their diameters. Follicular fluid (FF) contents were individually aspirated and centrifuged. Supernatants were separately collected and estradiol (E2) levels were analyzed by ELISA. Samples with highest E2 contents within the same group were pooled for protein determination, and analyzed for global proteome profiles using a LC-MS/MS. Experiments were repeated three times. Fluids originated from LF contained higher amounts of estradiol compared to MF (1.8x on average). In each sample, over 2,000 proteins were identified, in which approximately 22% were predicted and 12% were partially annotated. Lower proportions of proteins were found differentially accumulated ($P<0.05$; 7%), and 3.2% had higher significance ($P<10-5$). Moreover, 1.4% and 1.2% of proteins appeared specific to MF-FF and LF-FF, respectively. We concluded that FF is rich in proteins which differential detection and stage-specificity could help define potential markers of occyte quality.

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Smooth Muscle Hypertrophy is a Histologic Feature of Airway Remodeling in Summer Pasture Obstructive Pulmonary Disease. C. Bowers*, N. Mujahid, L. R. Costa, C. Ferrari, J. Cooley, S. C. Burgess, and C. E. Swiderski

Despite prevalence in 3-5% of the US population, mechanisms responsible for airway hyper-responsiveness in chronic human asthma remain elusive. Neutralization of inflammatory mediators indicates that asthma pathophysiology is not explained solely by inflammation. Characteristics of asthmatic airway smooth muscle (ASM) including constitutive increases in velocity of contraction and correlation between increased ASM mass and asthma severity make ASM a major investigative focus. Rodents do not spontaneously develop asthma nor chronic airway remodeling, including increased ASM that characterizes human asthma. By contrast equine RAO is a naturally occurring, environmentally induced, asthma-like syndrome affecting an out bred population of animals. Two forms of RAO are described: an indoor form occurring in stalled horses in cooler temperate regions and a summer pasture-associated (SPA) form occurring in the southeastern US. Recent documentation of increased ASM in indoor RAO led us to hypothesize that increased ASM contributes to the bronchospasm that characterizes SPARAO. We evaluated this hypothesis using immunohistochemistry and histomorphometry to quantify ASM of 210 SPARAO and 175 control airways in archival lung samples from the diaphragmatic, apical and accessory lobes of 6 SPARAO (ages 10-20) and 6 control horses (ages 10-22). Comparison of ASM area to airway basement membrane perimeter ratios in SPARAO versus control using a two tailed t-test for samples of unequal variance confirms a significant increase ($P=0.004$) in ASM in SPARAO airways relative to controls [95% CI SPARAO: 18.1$\pm$ 2.4; control: 13.9 $\pm$ 1.5]. Further, we demonstrate that increased ASM in SPARAO affects the diaphragmatic and apical lobes but not the accessory lobe.

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

Comparative proteomics of cryopreserved boar spermatozoa. Z. A. Brown*, J. M. Feugang, R. M. Black, K. Pendarvis, S. Willard, and P. Ryan.

Semen cryopreservation is a useful tool in long-term storage of superior genetics and allows widespread use of these genetics without transportation of breeding stock. Unfortunately, extensive use of sperm cryopreservation is limited in the swine industry due to the susceptibility of sperm to freezing-thawing procedures and great variability within ejaculates and boars. Here, we characterized the sperm proteome of boars of known freezing

ability for the identification of potential protein candidates for semen freezability. Frozen semen from fertile boars known to be “good freezers” and “poor freezers” was acquired from a local boar stud. Semen was thawed and motile sperm were purified using a 45-90% discontinuous percoll density gradient via centrifugation. The pellet containing high motile spermatozoa was washed three times with a cold PBS-PVP buffer. For each group of boars (4x4), purified sperm samples from three separate ejaculates were pooled and analyzed for global proteome profiles using a LC-MS/MS. Over eighteen hundred proteins were identified in each group, with proportions of 24% and 34% of total proteins found predicted or partially annotated in “good” and “poor” freezers' groups, respectively. Approximately 8% of total proteins were differentially expressed between both groups ($P<0.05$). The fold change of 2.4% of total proteins were significantly higher ($P<10-5$), and could be used as potential candidate markers of boar semen freezability. This study provides a unique set of protein markers that could serve to improve cryopreservation techniques of pig semen. However, further studies are needed to evaluate the significance of the selected proteins.

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Determination of Antimicrobial Susceptibility Profiles of *Salmonella* Isolates from Various Agricultural and Environmental Sources. A. Daniel*, R. H. Bailey, J. Brooks, R. Wills.
Salmonella is an organism of interest to the food production industry due to regulatory constraints and the organism’s ability to cause disease in humans. The purpose of this study was to determine changes in antibiograms over the last decade in Salmonella isolates collected from different poultry production sites. Of 113 Salmonella isolates collected from poultry production, 19 were from 2000 to 2001, 42 were from 2003 to 2006, and 52 were from 2010. An additional 15 isolates were collected from various species from 1999 to 2000. The Kirby-Bauer antibiotic disk diffusion method was used to test twelve antibiotics representative of the eight antibiotic classes. The results have shown that all antibiograms are relatively similar. All samples were resistant to penicillin, erythromycin, and bacitracin. All samples were susceptible to enrofloxacin, ceftiofur, neomycin, kanamycin. A cluster of 11 samples from the mid 2000’s tested resistant to gentamicin, oxytetracycline, tetracycline, cephalothin, or streptomycin. Overall, there has been little change in the antibiogram of various isolates of Salmonella over the time period tested.

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Proteomic identification of novel proteins in mare cervicovaginal fluid as early diagnostic biomarkers of equine placentitis. B. J. Evans*, J. M. Greene, D. L. Christiansen, and P. L. Ryan
Equine placentitis due to ascending bacterial infection is a leading cause of abortions and stillbirths in horses, which results in major economic losses to the horse industry. Early diagnosis of placentitis is essential in the treatment of the disease and prognosis of the pregnancy. Currently no diagnostic tests are available for the early detection of placentitis in mares, although recent advancements in molecular biology, namely proteomics, seem promising. In other species, several novel proteins from cervicovaginal fluid (CVF) have been indentified in preterm births and intra-amniotic infections. The objective of this study was to profile novel inflammatory proteins in CVF of pregnant pony mares using proteomics. CVF was collected from three uninfected controls, on days 310, 315, and 325 of gestation. Two mares were intracervically infected with 5 x 106 *Streptococcus equi* sub.*zooepidemicus*, which served as models of ascending placentitis. CVF of infected mares was collected on day 310 of gestation, before inoculation; then 24, 48, and 72 hours post inoculation. One mare aborted at 52 hours and the 72 hour sample was obtained post-abortion. The second mare aborted 103 hours post infection. The supernatant from samples was collected and stored at -70° F for proteomic profiling (results pending). The data obtained from this experiment may provide a profile of novel proteins that can provide early detection of equine placentitis. Early diagnosis and subsequent early treatment may reduce perinatal mortality in horses. Additional studies may enhance current knowledge of inflammatory processes and physiologic responses in at risk pregnancies.

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

Pharmocodynamic Assessment of the Immunosuppressive Effects of Cyclosporine versus Dexamethasone on Canine T-Cells using Flow Cytometry and Real-Time RT-PCR. R. Flores*, C. Fellman, J. Stokes, T. Archer, K. Lunsford, and A. Mackin

Cyclosporine and glucocorticoids are powerful immunosuppressive drugs which are used to treat inflammatory and immune-mediated diseases in dogs. Cyclosporine works by inhibiting the calcineurin-dependent pathway of T-cell activation, while glucocorticoids directly inhibit the genes that code for cytokine production. Dosing of both agents is often empirical, and little work has been done comparing their effects on T-cell cytokine production in dogs. Our study is designed to assess these effects by measuring cytokine production using flow cytometry, and cytokine gene expression using real-time RT-PCR in activated canine T-cells treated with cyclosporine and dexamethasone. For flow cytometry assays, peripheral blood mononuclear cells were separated using density gradients and cultured for 12 hours in the presence of cyclosporine, dexamethasone, or cyclosporine plus dexamethasone. Whole blood was cultured for 5 hours with the same treatment groups, and RNA was extracted from leukocytes for real-time RT-PCR. The expression of cytokines IL-2 and IFN-γ was analyzed in resting and PMA/ionomycin-activated T-cells by flow cytometry, and gene expression for IL-2 and IFN-γ in activated cell populations was also assessed. Flow cytometry and real-time RT-PCR showed dose-dependent inhibition of IL-2 and IFN-γ in response to both cyclosporine and dexamethasone. Suppression of the cytokines IL-2 and IFN-γ in activated T-cells has potential as an indicator of the efficacy of cyclosporine and glucocorticoid therapy at suppressing T-cell function *in vivo* in dogs, and may therefore be of value for characterizing the immunosuppression induced by these drugs in clinical patients.

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Potential role of turtles in the introduction of parasitic larval nematodes (*Serpinema trispinosum*) in commercial catfish. M. R. Harlow*, C. Panuska, L. M. Pote, and F. Austin

Commercial catfish ponds not only provide an ideal ecosystem for catfish, but also for wildlife. Many of these wildlife species are infected with parasites that can have fish as hosts in their life cycle. Recently, larval nematodes that were found encysted in commercial catfish muscle were identified as dracunculoids, some of which have turtles as their definitive host. Five catfish ponds, from two farms, with a history of dracunculoid nematode infections in the fish population were selected (Farm A: 2 ponds; Farm B: 3 ponds) to determine if turtles were positive for dracunculoid infections. Twenty -four red eared sliders (*Trachemys scripta elegans*) were collected, transported to the CVM-MSU, weighed, measured, and necropsied. Parasites were collected and enumerated. Invertebrates were also collected and microscopically examined for larval nematodes. Although dracunculoids were not found, another parasite that is known to have a fish host, *Serpinema trispinosum,* was found. The prevalence of *S. trispinosum* ranged from 80% (Farm B: 2/3 ponds) to 100 % (Farm A: 2/2 ponds; Farm B: 1/3ponds). Infected turtles averaged worm counts of 59-79 worms/turtle (range: 0-400). A large portion (1654 bp) of the 18S rRNA gene of this parasite was sequenced and found to be 98% homologous with the spirurid *Camillanus oxycephalus*. This research confirmed that turtles in catfish ponds are infected with at least one nematode, *S. trispinosum,* which has the potential to infect catfish. The sequencing data generated in this study will be used to develop molecular tools to detect other life stages of this parasite.

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Prevalence of Borrelia burgdorferi in *Sigmodon hispidus* (cotton rat) in selected areas of Oklahoma, Texas, and Mississippi. D. Link*, G. Moraru, and A. Varela-Stokes

Lyme disease is a tick-borne disease caused by the bacterial agent *Borrelia burgdorferi*. In untreated cases the

disease may lead to widespread rashes, joint, heart, and nerve inflammation, and changes in mentality. Although Lyme is not endemic in the southern United States, its lower prevalence here warrants a better understanding of the natural history, including which vertebrates may be used as reservoir hosts. The susceptibility of *Sigmodon hispidus* (cotton rat) to *B. burgdorferi* and the cotton rat's distribution throughout the southern U.S. has been documented and led to our study objective to determine prevalence of *B. burgdorferi* in cotton rats from selected areas of the South. A total of 143 whole blood and kidney tissue samples were obtained from cotton rats in Mississippi, Texas, and Oklahoma. Nested and heminested PCR assays were performed to detect *Borrelia* spp. in DNA extractions using genus-wide *flaB* and *16S rRNA* gene targets, respectively; positive samples were sequenced. In addition, available sera was tested (n=7) from Mississippi samples for antibodies to*Borrelia* spp. DNA from one blood sample from Mississippi was PCR positive for *Borrelia* sp. *flaB* only. The *flaB* sequence was 100% identical to sequences published in the National Center for Biotechnology Information (NCBI) for *B. burgdorferi* sensu stricto. All other samples were negative. Although prevalence was low, the positive sample suggests that cotton rats in the South may be infected with *B. burgdorferi* and play a role in the maintenance and transmission of *B. burgdorferi*here.

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Kinetics of wound infection and vaccine potential of four mutant strains of the catfish bacterial pathogen *Edwardsiella ictaluri*. K. McDaniel*, S. Menanteau-Ledouble, and M. L. Lawrence.
Edwardsiella ictaluri is the causative agent of enteric septicemia of channel catfish (ESC), a gastrointestinal septicemia affecting juvenile catfish in the spring and fall. ESC can cause mortalities up to 100% and is considered one of the most economically devastating diseases in farm-raised catfish. *E. ictaluri* is a facultative intracellular pathogen and is resistant to phagocytosis. Furthermore, despite the availability of a commercial vaccine, the disease is still one of the most prevalent in the industry. We previously identified *E. ictaluri* mutants that do not colonize catfish skin efficiently, and we hypothesize that these mutants could be effective live attenuated vaccines for protecting fry against ESC. Therefore, in the present study, the bacterium's invasion following topical application on sites of skin abrasion was recorded for four of these mutant strains and compared to that of the wild type. Results from this challenge suggested that, for most mutant strains, colonization of the abrasion site was indeed impaired. A second experiment was conducted during which these strains were used in a vaccine trial on 14 day old fry, followed four weeks later by exposure to a wild type strain of *E. ictaluri*. Mortalities were recorded daily for 30 days and compared to that of sham-vaccinated fry. Results from this experiment suggested that while the mutants were not as attenuated in fry as they were with older fish, several of them did provide some degree of protection, making them potentially interesting vaccine candidates.

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Effect of in vivo reduction of Nurr1 expression in Nurr1-null heterozygous (+/-) mice on nigrostriatal dopamine neurotransmission and dopamine neuron survival. S. McShane*, J. B. Eells, and S. Guo-Ross.
Parkinson's disease is a neurodegenerative disorder characterized by the permanent loss of dopaminergic neurons in the substantia nigra and subsequent reduction of dopamine in the striatum. The transcription factor Nurr1 is expressed in developing mesencephalic dopamine neurons and throughout adult life. In the absence of embryonic Nurr 1, dopamine neuron precursor development and expression of dopamine neuron specific proteins is arrested. Most studies have focused on the role of Nurr1 function in dopamine neuron development. Less is known about how Nurr1 regulates dopamine function/survival in adult dopamine neurons. Subsequent studies have shown that Nurr 1 levels in dopamine neurons decline with age. Our hypothesis is that if the expression of Nurr1 in dopamine neurons is reduced with age, then Nurr1 +/- mice, which already have reduced Nurr1 function, will show greater decline in dopamine levels and neuron survival with aging. Furthermore, morphologic changes such as loss of dopamine neurons cell bodies and/or synapses in target regions are also expected. To test part of this hypothesis, the current experiment used unbiased stereology to estimate the number of dopamine neurons in the substantia nigra and ventral tegmental area of young (3-4 months) and old (>15months), +/+ and +/- mice. The preliminary data showed there was a reduction of dopamine neurons in old

+/- mice as compared to young +/- mice. These data suggest an important role for Nurr1 in the survival of dopamine neurons and may be an important target for pharmacotherapeutic treatment of Parkinson's disease.

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The effect of a mock cold-smoking treatment on the intracellular viability of *Listeria monocytogenes*. C. Miller*, J. R. Pittman, and J. R. Donaldson
Listeria monocytogenes is a very dangerous food-borne gram-positive bacterium that is the causative agent of listeriosis. Smoked ready-to-eat products, such as smoked salmon, have a higher prevalence of being contaminated with *L. monocytogenes*. Sources of contamination proposed by others in cold-smoked products include: persistent strains present in facilities, poor handling by workers, and/or contaminated fish entering the facility. Previous studies from this lab have found that the cold-smoked process might precondition virulent strains to be more resistant to downstream storage conditions as compared to avirulent strains of *L. monocytogenes*. In order to determine whether the cold-smoking process and subsequent storage affects the pathogenic potential of the microbe, virulent and avirulent strains of *L. monocytogenes* were exposed to either a mock cold-smoking treatment or to media only and subsequently infected in the human adenocarcinoma HT-29 cell line. Intracellular growth was then monitored over a 5-hr period by viable plate counts. We found that for the avirulent strain ATCC15313, as well as for the virulent strains HCC7 and F2365, there was no significant difference in intracellular growth. We found that the intracellular viability of the avirulent strain HCC23 was significantly decreased following exposure to the mock cold-smoking process. These data indicate that the cold-smoking process does not precondition *L. monocytogenes* to be more resistant.

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Competitive Evolution between the Co-infected H1N1 and H3N2 Human Seasonal Influenza A Viruses. D. L. Russell* and H. Wan.
Influenza A virus causes both pandemic and seasonal influenza epidemics. It is not uncommon to detect the same subtypes of influenza A virus in the same influenza outbreaks. A previous study showed that less than 5% of patients could be co-infected with both the H1N1 seasonal influenza A virus (A/H1N1) and the H3N2 seasonal influenza A virus (A/H3N2) while the others were infected only by either A/H1N1 or A/H3N2 virus. The underlying molecular mechanism for such a dynamic is still not clear. We hypothesize that there is a competitive evolution between the co-infected influenza A viruses. The goal of this project is to study the competitive dynamics of A/H1N1 and A/H3N2 viruses in temporal order when these viruses co-infect the cells. This study is performed *in vitro* using Madin-Darby Canine Kidney (MDCK) cells. Three groups of experiments were designed: (1) the same amount (in pfu) of A/H1N1 and A/H3N2 viruses co-infect MDCK cells at the same time; (2) A/H1N1 virus infects MDCK cells 2 hrs before the A/H3N2 infection; (3) A/H3N2 virus infects MDCK cells 2 hrs before A/H1N1 infection. The HA and NA genes of both viruses were quantified using real-time PCR at time 0, 2, 4, 8, 12, 24, and 72 hrs after the infections. The quantitative dynamics of A/H3N2 and A/H1N1 viruses are explored. This project will help us understand the molecular mechanisms during influenza viral co-infection

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An immunohistochemistry assay to detect regulatory T cells (Tregs) in frozen feline placental tissue. S. L. Tankersley*, C. E. Boudreaux, and K. S. Coats.
Regulatory T cells (Tregs) are important mediators of the immune system and play a role in the maintenance of the proper immunological balance necessary for successful pregnancy. Tregs are known to suppress both CD4+ and CD8+ T cells and thus inhibit placental inflammation that may lead to fetal death. An increased occurrence of reproductive failure occurs in queens experimentally infected with FIV, an important small-animal model for HIV transmission and pathogenesis. We hypothesized that FIV infection may alter Treg population dynamics

and function in the placenta. The aim of this study was to develop an immunohistochemistry (IHC) assay to identify Tregs specifically in placental tissues for gene expression analyses. Tregs were labeled using rabbit polyclonal antibody to FoxP3 in an IHC assay, then the cells were selectively removed from the tissues using laser capture microdissection. RNA was purified from the cells, and expression of FoxP3 was measured using real time PCR. The IHC assay specifically identified Tregs in the placental tissues by targeting their expression of FoxP3 protein. Real time RT-PCR confirmed expression of FoxP3 transcripts. These techniques will be used in studies of the effect of FIV infection on placental Treg function, potentially advancing the understanding of placental immunolopathology in HIV-infected women that may predispose vertical transmission and/or reproductive failure.

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Expression of the EphB2 receptor and ephrin-B1 ligand in the bursa of Fabricius. B. Weathers*, S. L. Branton, E. D. Peebles, R. L. Taylor, R. Jacob, and G. T. Pharr

Chicken B-cells develop in a specific organ, the bursa of Fabricius. In the embryonic period, a key differentiation event occurs which initiates the process of repertoire development by immunoglobulin diversification between embryonic days (ED) 15-18. To understand the bursal microenvironment guiding B-cell differentiation in the embryonic bursa, previous studies revealed the expression of the ephrin (Eph) receptor B2 subfamily Eph receptors. Eph receptors and ephrin ligands direct the organization of cells within tissues and control cellular differentiation in both embryonic and adult tissues of avian and mammalian species. We hypothesize that Eph receptors and their ligands regulate the critical cell contacts between developing B-cells and stromal cells in the embryonic bursal follicle. To address this hypothesis, we need additional basic studies on the observation of EphB2 expression and to determine if its ligand, ephrin-B1, is expressed in the embryonic bursa. Therefore, the objective of this project was to examine *EphB2* and *ephrin-B1* gene expression with reverse transcriptase-PCR and Western blotting in the bursa at ED15 and ED18. The RT-PCR experiments with bursal cDNA amplified transcripts from the *EphB2* and *ephrin-B1* genes, which were confirmed by nucleotide sequencing. The anti-EphB2 and anti-ephrin-B1 antibodies recognized proteins of the expected molecular mass (120 and 55 kDa, respectively) and gave an acceptable signal to noise ratio on Western blots of whole tissue proteins from the ED15 and ED18 bursa. This information can be used to design experiments for determining the cell type(s) expressing the EphB2 and ephrin-B1 proteins in the embryonic bursa.

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