Student Abstracts 2009

Trainees

Front row (L to R): Alexandria Murphy, Todd Sullivan, Kim Pluta, Missy Taylor, Cassie Bayer, Cyprianna Swiderski (faculty mentor)
Second row (L to R): Claire Fellman, Matt Castellaw, Drew Leach, Lukina Burks
Third row (L to R): Danielle Chemerys, Henderson King, Jesse Grady, Grant Ramsey
Fourth row (L to R): Autumn Harris, Brian Rakauskas, Dr. Hart Bailey (faculty mentor), Dr. Mark Lawrence (faculty mentor)
Fifth row (L to R): Ryan Centini, Youry Dupre, Dr. Larry Hanson (faculty mentor)
Sixth row (L to R): Dr. Camilo Bulla (faculty mentor), Dr. Andrea Varela-Stokes (faculty mentor)
Not pictured: John Burnett

Evaluation of two anti-thrombopoietin antibodies for cross-reactivity with canine thrombopoietin.
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Thrombocytopenia is a common pathologic condition in dogs. It occurs in many diseases and is caused by several mechanisms. These mechanisms include blood loss, sequestration, consumption, destruction, and decreased production. The latter is frequently associated with a poor prognosis. Platelet production is mostly regulated by thrombopoietin (TPO), a cytokine with a highly conserved N-terminal domain, where its binding site is located. TPO is normally produced constitutively by the liver and circulating levels are regulated by internalization and degradation after binding to the receptor, Mpl, that is present mostly on megakaryocytes and

platelets. Circulating levels of TPO have been used as a reliable marker for assessing platelet production in several species. In dogs, the platelet production assessment, currently based on bone marrow biopsy evaluation by a trained pathologist, is time consuming, invasive, and usually yields a subjective result. Currently, there is neither an available test validated for canine TPO measurement, nor a commercially available anti-TPO antibody shown to bind to the canine TPO. With the objective of developing and validating an ELISA for assessing canine TPO concentration in serum, two commercially available polyclonal antibodies, anti-murine and anti-human N-terminal TPO, were tested in a Western Blot for cross-reactivity with recombinant canine N-terminal TPO. The results suggest that these two antibodies cross-react with recombinant canine N-terminal TPO and are good candidates for the development of the ELISA to measure circulating levels of TPO in dogs.

Dual Potency of Anti-HER2/neu and Anti-EGFR Anthracycline-Immunoconjugates in Chemotherapeutic-Resistant Mammary Carcinoma Combined with Cyclosporin-A and Verapamil P-glycoprotein Inhibition

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Malignant mammary carcinoma originates from within the secretory and ductal tissues of the breast. In women affected by neoplastic disease, breast cancer is second only to lung cancer as the leading cause of mortality. The most aggressive forms of breast cancer that are chemotherapeutic-resistant often over-express P-glycoprotein and epidermal growth factor HER2/neu and EGFR receptors. Collectively, expression of these membrane associated complexes directly influences cancer cell biology pertaining to proliferation rate, differentiation and potential to metastasize to distant tissues and organ systems. In an effort to improve efficacy against aggressive chemotherapeutic resistant mammary carcinoma and reduce the side effects associated with conventional chemotherapy the anthracyclin, epirubicin was covalently linked to anti-HER2/neu and anti-EGFR monoclonal immunoglobin. The synthetic methodology involved the application of the heterobifunctional covalent linking agent, succinimyl-4-(N-maleimidomethyl)-cyclohexane-1- carboxylate with was introduced at the - monoamide group of epirubicin to create a sulfhydryl-reactive intermediate. In another stage of synthesis, succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate was used to introduce sulfhydryl groups into immunoglobulin at the terminal amine of -lysine residues. Epirubicin-(anti-HER2/neu) and epirubicin-(anti-EGFR) had greater potency against chemotherapeutic-resistant mammary carcinoma than equivalent epirubicin concentrations. Epirubicin-(anti-HER2/neu) bound to a greater extent to the surface membrane of mammary carcinoma cells due to greater HER2/neu expression densities more EGFR and was therefore more potent than epirubicin-(anti-EGFR). Combined epirubicin-(anti-HER2/neu) and epirubicin-(anti–EGFR) 50/50 combinations produced synergistic levels of anti-neoplastic activity.   Competitive P-glycoprotein pump inhibition with cyclosporine-A or verapamil enhanced epirubicin-immunoconjugate potency. The investigations represent potential strategies for enhancing the selective internalization, intracellular deposition and anti-neoplastic potency of chemotherapeutics in multi-drug resistant neoplasias.

Antimicrobial Activity of Avian Beta-Defensin 12

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Multiple tissue types in many mammalian species contain defensins, which are cationic cysteine-rich peptides that kill microbial pathogens and trigger an immune cell response to the site of infection. The cysteine residues are connected by di-sulfide bonding. Alterations in the bonding arrangements are the basis for α-, β-, and θ-

classifications of defensins. In chickens, 14 different Avian β-Defensins (AvBD) have been found in tissues with frequent contact to pathogens, including the Respiratory tract and the Urogenital tract. AvBD-12 is expressed in elevated levels in the chicken oviduct epithelial cells, but the biological functions have not been established. In this study, the antimicrobial capabilities of synthetic AvBD-12 (sAvBD-12) and recombinant AvBD-12 (rAvBD-12) against food borne bacteria were determined using a modified micro-broth dilution method with log-phase and stationary-phase bacteria. Our results show that sAvBD-12 was more effective against *Staphylococcus aureus* and *Listeria monocytogenes* than *Salmonella typhimurium* and *Escherichia coli*. The rAvBD-12 also showed high effectiveness against *Staphylococcus aureus*. At levels of 8 μg/ml, 16 μg/ml, and 32 μg/ml, the effectiveness levels of the synthetic and recombinant against *Staphylococcus aureus* were comparable. At 64 μg/ml, sAvBD-12 was significantly more effective against *Staphylococcus aureus*. In conclusion, we see that AvBD-12 has more antimicrobial capability against Gram-positive bacteria than Gram-negative. Future studies are needed to establish sAvBD-12 as a possible supplement for ensuring ovulation and increase rates of breeding success. Further investigation is also needed to determine possible food safety and public health benefits of sAvBD-12 and rAvBD-12.

Use of Biophotonics as a Real Time Model to Determine the Role of Filth Flies in the cross contamination of cattle with *E. coli* O157:H7

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 A possible mechanical vector for *E.coli* O157:H7 contamination of cattle and food products is filth flies. Biophotonics provides a real time model for monitoring this pathogen within the life cycle of the filth fly. The objective was to determine if larvae from filth flies could ingest *E.coli* utilizing a biophotonic transformed *E.coli* XEN 14 (**BP-*E.coli***) transformed with the pAK1-lux gene cassette. Larvae were incubated in 50- ml conical tube containing 8-g of sterilized bovine manure inoculated with BP-*E.coli* (2 X 109) for 24 or 48-h. Post-incubation, larvae were imaged to determine uptake of BP-*E.coli*. Post-imaging, larvae were macerated, re-imaged, and serially diluted to quantify total CFU's of BP-*E.coli* ingested. Data were analyzed using PROC GLM procedures of SAS ($P<0.05$). Imaging of intact larvae exposed to BP-*E.coli* for 24 and 48-h revealed that 76% and 56% of the larvae had ingested BP-*E.coli*, respectively. Serial dilution indicated that 86% and 80% of larvae had ingested BP-*E.coli* at 24 and 48-h, respectively. For 24-h incubation, larvae were able to ingest 1 X 106 CFU, after 48-h incubation larvae were able to ingest 1 X 105 CFU. Results of this preliminary trial indicate that larvae of filth flies could ingest *E.coli* O157:H7. Further research is being conducted to determine if the BP-*E.coli* is viable during and after the pupil stage and emerge with the adult fly; and if so, how much *E.coli* can be deposited by filth flies, thus serving as a vector for contamination of cattle and food products.

Cardiac findings in American Quarter Horses with Hereditary Equine Regional Dermal Asthenia

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Hereditary equine regional dermal asthenia (HERDA) is an autosomal recessive disorder of Quarter Horses caused by a mutation that affects collagen folding. The skin of affected horses is loose, hyperextensible, and

fragile. Affected horses develop lacerations, hematomas, and disfiguring scars from minor trauma. Cutting horses are most commonly affected; 14 of the top 100 cutting sires are carriers with offspring earnings in excess of $90 million. Clinically similar connective tissue dysplasias are seen in a wide array of species and include Ehlers-Danlos Syndrome (EDS) in humans. Humans with EDS can have problems with tissues other than skin including joints, ocular structures, and the cardiovascular system. EDS affects 1 in 5000 individuals and can cause significant morbidity and even early mortality in those affected. To evaluate the effect of HERDA on the cardiovascular system, affected horses underwent echocardiographic evaluation of their hearts and great vessels. Biomechanical test were then performed on the aortic and mitral valves and ultimate tensile strength was compared to control horses. Affected horses have a high prevalence of aortic valve insufficiency. In the biomechanical study the aortic valves of HERDA horses were significantly weaker than normal horses in both the radial ($p=0.0007$) and circumferential ($p=0.001$) directions. These results further support the potential use of HERDA as an animal model of human disease.

Channel Catfish Virus: Biological and antigenic diversity

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Ictalurid herpesvirus 1 or channel catfish virus (CCV) is a member of the Alloherpesviridae family of the Herpesvirales order. It is a large enveloped virus that has a linear, 143 kilobase pair (Kbp) double- stranded DNA genome. CCV causes a sporadic outbreak of acute hemorrhagic disease amongst catfish fry and fingerlings which occurs more frequently in warmer temperatures. Although extensive molecular characterization of the type strain of virus has been done including the genome sequences, little has been done in investigating the genetic and biological diversity of field isolates of CCV. We evaluated 24 field sample isolates from various locations in the southeast United States to determine the genetic and antigenic diversity of CCV. The field samples were grown in Channel Catfish Ovary (CCO) cells and their DNA extracted for use in Restriction Fragment Length Polymorphism (RFLP) using *Eco*R1, *Kpn*1, and *Xho*1. These samples were also used in neutralization assays with a neutralizing monoclonal antibody to determine antigenic variation. Genetically the banding patterns for the FRLP are showing vast similarities with few differences to the other CCV isolates within the group. However, compared to the type isolate, Auburn-1, the tested field isolates show more frequent differences. The genetic and antigenic diversity detected will indicate the utility of impact antibody and genetic based detection methods for disease diagnosis and will help determine diverse strains for use to evaluate efficacy of vaccines.

Assessing Heat Load and Dissipation Using Digital Infrared Thermography and Serum Cortisol Profiles in Horses During the Summer Months

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The objective of this study was to evaluate heat load in horses during the summer months and whether coat color is a factor. Fifteen mares were assigned to one of five coat color categories (n= 3/group; bays, browns, grey, paint, palomino) and maintained on pastures with the same shade cover. Heat load was assessed using thermal imaging (FLIR T400 camera) and rectal temperatures were recorded 4x/d (0600, 1200, 1600, 2000hours), 2 d/week for six weeks (June 1-July 15, 2009) along with ambient temperature, wind speed and

humidity. Five regions of interest (ROI) (flank, shoulder, eye, muzzle, perineum) were imaged at each time point. Minimum (MIN), maximum (MAX), average (AVE), and standard deviation (STDEV) thermal signatures were assessed for each ROI. Blood was collected at 0600, 1600 and 2000hours for serum cortisol to asses heat stress. Data was analyzed using GLM procedures of SAS. Rectal temperatures were similar for all groups regardless of time points. Bay and brown horses had the highest thermal signature (AVE/MAX) at 1200 and 1600 h in all ROIs except the perineum and eye compared to other groups ($P<0.05$). Shoulder and flank regions had the highest AVE/MAX (~40˚C) compared to other ROIs. Grey mares had the greatest perineum temperature (37.15-37.7˚C) throughout the day ($P<0.05$) compared to other groups (34.32-37.56˚C). In conclusion, coat color affects heat load and dissipation in horses, which is influenced by the darker color consistent with heat absorption. Serum cortisol profiles will be correlated with thermal signatures to determine level of heat stress.

Cyclosporine A Affects the *In Vitro* Expression of T Cell Activation-Related Molecules and Cytokines in Dogs

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Cyclosporine is a powerful immunosuppressive drug that is being used with increasing frequency to treat a wide range of inflammatory diseases in the dog. To date, ideal dosing protocols that will achieve immunosuppression with cyclosporine in dogs remain very unclear, and standard methods that can measure effectiveness of immunosuppression have not been established. The aim of our study was to evaluate the effects of *in vitro* cyclosporine on a panel of markers expressed on activated T cells to ascertain their potential as biomarkers of immunosuppression in dogs. Blood was drawn from three healthy dogs, and peripheral blood mononuclear cells were isolated and activated. Half of the cells were incubated in the presence of 200 ng/mL cyclosporine and the other half were untreated. Samples were analyzed using flow cytometry, and the expression of intracellular cytokines IL-2, IL-4, and IFN-γ was evaluated after 6, 12, and 24 hours of drug exposure. Each cytokine exhibited a time-dependent suppression profile, and all samples activated in the presence of cyclosporine showed much lower cytokine expression than untreated controls. We also evaluated the expression of the surface T cell activation molecules CD25 and CD95 via flow cytometry after 36 hours of drug exposure. Expression of these surface molecules decreased when activated in the presence of cyclosporine. Our results suggest that suppressed expression of the markers related to T cell activation could be used as an indicator of the efficacy of cyclosporine therapy in dogs.

Effects of Cyclosporine on Canine Platelet Activation

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Cyclosporine is an increasingly popular immunosuppressant drug used for the treatment of canine immune-mediated diseases. Immune-mediated hemolytic anemia (IMHA) is one of the most common causes of anemia in canine patients. The mortality rate of IMHA patients can be as high as 70%, with pulmonary thromboembolism being the most common cause of death. Recent investigations have demonstrated that cyclosporine may increase platelet procoagulant activity in humans. Because IMHA dogs are already predisposed to thrombus formation, the administration of cyclosporine could potentially increase the likelihood of forming a pulmonary thromboembolism. In our study, we evaluated the effects of cyclosporine on canine platelet expression of

activated platelet-specific markers P-selectin and phosphatidylserine by flow cytometric analysis. Cyclosporine was administered orally at standard immunosuppressive doses to 8 healthy dogs twice a day for one week, and samples were obtained at peak and trough serum drug concentrations immediately after initiating therapy, and after 1 week of drug administration. Flow cytometric analysis revealed a 16% decrease in platelet P-selectin expression and a 42% decrease in phosphatidylserine expression compared to baseline levels. The decrease in expression of activated platelet surface markers suggests that although the use of cyclosporine in dogs appears to affect platelet surface membranes, the drug does not appear to elicit an increase in platelet procoagulant activity. Based on our results, the administration of cyclosporine to dogs may not cause a platelet procoagulant state, and therefore does not contribute to the development of pulmonary thromboemboli if administered to canine IMHA patients.

Involvement of Phosphatidylinositol 3-Kinase Pathway in *Salmonella Kentucky* uptake in bovine monocytes

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Monocytes are professional antigen presenting cells (APC) that are capable of internalizing bacterial pathogens by phagocytosis and presenting antigen to immunocompetent lymphocytes. Previous reports demonstrated that LPS, a component of the outer membrane of Gram-negative bacteria, in particular, *Salmonella,* stimulates the toll-like receptor 4 (TLR4) signaling in monocytes/macrophages that results in rapid production of inflammatory mediators and cytokines. Recent reports showed that TLR4 triggering that is mediated mainly through the activation of the NF-B and MAPK signaling cascades also induces activation of Phosphatidylinositol 3-Kinase (PI3K) pathway that is involved in negative regulation of IL-12 and inflammatory cytokines by dendritic cells and monocytes/macrophages in humans and mice. In this study we investigated the mechanisms of *Salmonella Kentucky,* a mildly virulent intracellular pathogen, uptake in bovine monocytes in the presence of PI3K inhibitor wortmanin by Flow Cytometry. *Salmonella Kentucky* strain was isolated from chicken and was transformed with a plasmid expressing Green Fluorescent Protein (GFP). Our data indicate that live and heat-inactivated*Salmonella Kentucky* endocytosis was enhanced in bovine professional APC in the presence of wortmanin suggesting that PI3K pathway negatively regulates *Salmonella Kentucky* endocytic uptake in bovine monocytes.

Evaluation of Sampling and Culture Methods for *Salmonella* Enumeration by Most Probable Number in Broiler Carcass Rinses

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Salmonella, a member of the family *Enterobacteriaceae*, is an enteric borne gram-negative bacteria causing disease in both man and animal species. Increased regulatory pressure in the poultry industry, relative to the presence of *Salmonella* in the processing environment, has created the need for scientifically sound information for making logical risk management decisions pertaining to microbial control. The development of techniques to do risk factor analysis in the production environment requires the use of methods that are the most sensitive and accurate for both sampling and culturing of*Salmonella*. A primary focus of our laboratory is to fully characterize the ecology of the immersion chill tank in order to optimize its operation in controlling *Salmonella* contamination in the processing of broilers. The purpose of the work reported here was to determine the most appropriate

procedure for sampling carcasses in the processing plant, and then for disposition of the carcass rinse samples by culturing using the Most Probable Number Modified Secondary Enrichment Method. The effect of freezing on low numbers of *Salmonella* prior to culturing was determined using spiked samples. The information obtained in this work is a critical component of a larger project which is to identify the interaction between *Salmonella* and *Campylobacte*r levels on broiler carcasses prior to entering and upon exiting the immersion chill tank with parameters in the water including, dwell time, pH, chlorine, turbidity, re-dox potential and temperature.

The role of internalin-like proteins and other potential virulence factors in Listeria monocytogenes invasion of gut epithelium

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Listeria monocytogenes is a gram positive, opportunistic bacteria that is an important source of food-borne disease. Despite being treated as a pathogenic organism, the virulence of these bacteria can vary greatly between strains. One possible reason for this variability is the presence of internalins and other binding proteins in some strains that do not exist in others. The aims of this study were to: 1) use human colonic cell lines to explore the role of internalins in *L. monocytogenes* adherence of intestinal cells, and 2) clone and transfer two additional genes , *lmo0338* and *lmo0050*, from a pathogenic *L. monocytogenes* strain, F2365, into a non-pathogenic strain, HCC23, to determine if these genes contribute to pathogenicity. The adherence assay showed that F2365, the pathogenic strain, had significantly higher adhesion than any of the other strains. There was very little difference seen between HCC23 and either HCC23::*lmo2470* or HCC23::*inlC*. This suggests that these genes alone do not significantly increase adherence to gut epithelium. The HCC23::*lmo2821* strain produced results that, while not statistically significant (P=.059), merit future study to further explore the effect this gene has on adherence. Genes *lmo0338* and *lmo0050* were successfully amplified from F2365 and cloned.

Evidence of the tick-borne agents, *Borrelia lonestari* and *Ehrlichia chaffeensis* in migratory and resident song birds from Mississippi

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Amblyomma americanum ('Lone star tick'; LST), is an aggressive ixodid (hard) tick prevalent in the southern United States. While historically considered unimportant, it was recently recognized as a significant vector for disease, with the potential to transmit zoonotic bacterial pathogens, most importantly, *Ehrlichia chaffeensis*, agent of human monocytic ehrlichiosis. LST are also associated with "southern tick-associated rash illness", a Lyme-like syndrome potentially caused by *Borrelia lonestari*. The purpose of this study was to use serologic testing (immunofluorescent antibody testing; IFA) and the Polymerase Chain Reaction (PCR) technique to determine if wild passerines in Mississippi haveantibodiesto *E. chaffeensis* and *Borrelia* spp.or possess evidence of circulating organism. To date, we have collected heparinized blood samples from 47 birds of various species from Northern Cardinals and Mockingbirds to other, less common species. We extracted DNA from a portion of the sample and tested using nested PCR with primers to amplify the *flab* gene for *Borrelia* species and 16S rRNA gene for *E. chaffeensis*. Plasma was screened by IFA for antibodies to *E. chaffeensis* and *B. lonestari,* where our cut-off titer was 64*.* Our PCR results thus far show that 2/28 (7.14%) were *E. chaffeensis* positive and 0/8 (0%) were *Borrelia spp.* positive. IFA testing revealed 10/49 (20.4%) were seropositive for *E. chaffeensis* and

1/49 (2.0%) were seropositive for *B. lonestari.* These results show evidence of tick-borne agents in Mississippi birds and suggest birds have been previously exposed to and may harbor these tick-borne agents in nature.

Inhibition of human KIAA1363, a dual-role enzyme, by bioactive metabolites of organophosphate (OP) pesticides

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Serine hydrolase KIAA1363 is highly expressed in cancer cells, mouse brain, and human macrophages. It can metabolize low levels of oxons, bioactive metabolites of OP insecticides, and endogenous lipids (cholesteryl esters and acetyl monoalkylglycerol ether). The goal of this study was to express human KIAA1363 in COS7 cells in order to study its biochemical activity toward OP metabolites and other ester-containing substrates. In addition, we wanted to compare KIAA to the well-characterized OP scavenger, carboxylesterase 1 (CES1). We began by transfecting KIAA and CES1 cDNA into COS7 cells. Production of active enzymes was verified by treating cell lysates with an activity-based probe, fluorophosphonate(FP)-biotin. Hydrolytic activities of lysates toward a model ester substrate, p-nitrophenyl valerate (pNPV), were determined. The specific activities of KIAA- and CES1-transfected cells were 2- and 33-fold greater than mock-transfected cells, respectively, which indicated that pNPV was not hydrolyzed effectively by KIAA. To examine inhibition of KIAA by chlorpyrifos oxon (CPO), we pretreated protein with increasing concentrations of CPO (0.01-20µM) followed by addition of 2mM FP-biotin. FP-biotin–modified KIAA is recognized with avidin-horseradish peroxidase and detected as bands on film. However, if the active site of the enzyme is occupied by CPO, then FP-biotin is unable to covalently modify the protein. As CPO concentrations increased, FP-biotin–modified KIAA band intensity steadily decreased, indicative of enzyme inhibition. Following densitometry of bands, the half-maximal inhibitory concentration was estimated (IC50=20.5±1.5 nM). This indicates that KIAA1363 is sensitive to inhibition by CPO, although not to the degree of CES1 (IC50=0.5 nM).

Effects of a Proprietary Herbal Compound on Wound Healing Parameters and Bacterial Contamination in Horses

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Bioscreen, a proprietary herbal biocontamination product that inhibits bacteria, mold, and viral proliferation has recently been approved for cosmetic use in France. The purpose of this study was to determine whether Bioscreen, applied as a topical cream to wounds on the limbs of horses, can improve the rate of second intention healing and minimize bacterial contamination. To test this and determine the optimal concentration of Bioscreen for further studies of its efficacy in wound healing, wounds with a surface area of 6.25cm2 were created on horses under general anesthesia. Dilutions of Bioscreen topical cream (0%, 5%, 10%, and 25%) were applied to the wounds every other day for 3 weeks. Wounds were maintained under sterile bandages. Parameters of wound healing including perimeter, surface area, exudates, color, granulation tissue depth, and bacterial contamination were compared among wound treatment groups. Differences in healing parameters were evident between treatment groups. When compared to 0%, 5% and 25 % Bioscreen cream, 10% Bioscreen cream minimized wound surface area and perimeter on all days. 10% Bioscreen also improved wound appearance with wounds demonstrating a lighter pink color, less bleeding, and less granulation tissue in comparison to the 0%, 5%, and 25% cream treatments. Wounds treated with Bioscreen cream also lacked bacterial infection. We conclude that in comparison to creams containing 0%, 5%, and 25% Bioscreen, 10% Bioscreen improves wound

healing and optimizes wound appearance. Expanded studies on the benefits of 10% Bioscreen cream in comparison to medications commonly applied to equine wounds are warranted.

Use of quantitative real-time RT-PCR to confirm expressed protein sequence tags identified by proteogenomic mapping in *Mannheimia haemolytica*

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Mannheimia haemolytica is the primary bacterial pathogen contributing to bovine respiratory disease (BRD), a syndrome that causes extensive animal suffering and economic loss within the cattle industry. To understand the mechanisms of pathogenesis for *M. haemolytica,* description of its gene and protein expression under specific conditions is needed. To enable these studies, the protein-coding genes first have to be identified in the genome sequence. Currently, genes are identified using computer predictions. Proteogenomic mapping is a powerful complementary experimental method for identifying protein-coding genes in a genome as well as confirming and refining previously predicted genes from *in silico* analysis. A proteogenomic mapping pipeline developed by Mississippi State University was utilized to identify potential protein coding genes (expressed protein sequence tags or ePSTs) in *M. haemolytica* with associated confidence scores based on factors including start codons, conserved domains, and sequence homology. Our hypothesis is that a subset of highly ranked ePSTs from the proteogenomic mapping pipeline are expressed, and that their expression can be confirmed and quantified through the detection of individual RNA transcripts. Quantitative Real-Time PCR (qRT-PCR) was utilized to measure the expression of the ePSTs' RNA transcripts under specific growth conditions. The majority of the ePSTs analyzed did express RNA transcripts detectable by qRT-PCR. These results suggest that the proteogenomic mapping pipeline was successful in refining *in silico* predicted *M. haemolytica* genes and identifying sequencing errors in the draft genome*.*

Optimization of Targeted Photodynamic Therapy Against *Streptococcus canis*

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Multi-antibiotic resistant microbes are increasingly becoming a major global public health concern. Alternative treatment modalities have been developed to combat this threat. Photodynamic therapy (PDT) uses the combination of a photosensitizing agent along with harmless visible light to produce cytotoxic oxygen species that kill microbial and host cells. In the current study, Lumacare® was used as the light source with 5-aminolevulinic acid (5-ALA) being utilized as the photosensitizing agent. The goal of this study was to determine the shortest combination of pretreatment times with 5-ALA and light exposure for the most efficacious kill of common wound-infecting bacteria in vitro. *Streptococcus canis* from a clinical case in the Animal Health Center College of Veterinary Medicine Mississippi State University was used due to its prevalence in veterinary dermal wounds.. Optimal bacterial concentration was determined on 100-mm-diameter Remel® Blood Agar Plate to allow accurate colony counting using the Fisher Accu-Lite® colony counter during the experiment. The highest molarity solution of 5-ALA that had no decrease in viability for the bacteria was 0.1 millimolar (mM) with 15 minutes of pretreatment. This pretreatment time of 15 minutes at 0.1mM combined with 6 hours of light exposure reduced the colony count by 41%. To compare, placing the bacteria under the light alone for 6 hours reduced the colony count by only 19%. Thus concluding, that PDT is successful with a non-coherent light source to kill a common wound bacterium. However, clinical applicability may be limited by the long light exposure times needed in this model.

Determination of the Role of Nurr1in Regulation of Nigrostriatal Dopamine Neurotransmission

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The nigrostriatal dopaminergic systems, consisting of neurons in the substantia nigra pars compacta and innervating the striatum, is an important component of regulating basal ganglia control over motor function. It is the loss of these neurons that is the primary feature of Parkinson's disease, a progressive degenerative disease of the brain affecting approximately 1.5 million people in the United States. The nuclear receptor/transcription factor Nurr1 plays a key role in the dopaminergic system of the brain, essential for dopamine neuron development and survival and implicated in the regulation of dopamine synthesis. Currently, the role of Nurr1 in the regulation of nigrostriatal dopamine neurotransmission has not been established. To begin to assess the role of Nurr1 dopamine neurotransmission, the current study used microdialysis in the striatum to compare Nurr1-null heterozygous mice with their wild-type litter mates. With a microdialysis probe in the striatum, basal dopamine levels were measured (3 h) followed by stimulated dopamine overflow caused by a depolarizing concentration of potassium (100 mM K+ for 1 h) in the presence or absence of the D2 receptor agonist quinpirole (100 μM). Flow rate was 1 μl/min and fractions were collected every 20 min with a refrigerated fraction collector into 5 μl of 0.1M perchloric acid and 100 μM EDTA. Dopamine and the metabolites DOPAC and HVA were measured using HPLC with electrochemical detection. By comparing these parameters between Nurr1-null heterozygous mice and wild-type mice, we expect to determine the mechanisms through which Nurr1 may regulate dopamine neurotransmission.