

Abstracts from 2002 Program

Summary of Individual Projects: To guide students in selection of topics and preparation of proposals, the following summaries are abstracts of posters presented at the Annual Phi Zeta Research Emphasis Day on September 25, 2002. A list of Faculty who have expressed and interest in serving as mentors appears in the Appendix, although any faculty member can be selected for student projects. Further information on current individual faculty research programs can be obtained from SVM departmental web sites.

Determining the Antibiotic Resistance Patterns of Microbes Isolated from Wildlife Presented to the Wildlife Hospital of Louisiana (Baton Rouge, LA). E. Dahlgran, M.E. Mitchell, A. Roy, T. Tully. Veterinary Clinical Sciences, Louisiana State University.

To date, research into antimicrobial resistant bacteria has primarily focused on elucidating antimicrobial resistance patterns associated with human hospitals and livestock production, thus our understanding of the spread of antimicrobial resistant microbes into the environment is limited. The purpose of this study was to determine if wildlife presented to a wildlife rehabilitation facility could serve as a bioindicator for the environment in terms of antimicrobial resistant bacteria.

Cloacal of fecal samples were collected from all incoming adult wildlife to the Wildlife Hospital of Louisiana (WHL) in Baton Rouge using a sterile cotton tip applicator. Microbiological samples were also collected once a week from the WHL environment including the refrigerators, student hands and stethoscopes, cages, exam table, counter, floor, water, cold pack tray, restraint gloves, and air. The samples were immediately placed on 5% sheep blood and MacConkey agar and incubated for 48 hours at 37° under aerobic conditions. After incubation, distinct colonies were noted, transferred to stock agar, and Gram-stained. Gram-positive, coagulase positive *Staphylococcus* and Gram-negative rods were further evaluated using standard biochemical test media to characterize the isolates to the species level. Isolates from 5% sheep blood agar incubated for 18 hours at 37° under aerobic conditions were used for antimicrobial sensitivity testing. A sterile cotton tip applicator was then used to spread the inoculate of the isolate diluted to 0.5 MacFarland standard onto a Muller-Hinton agar plate. Eleven antibiotics were used in the sensitivity testing including Amikacin, Cefatoxime, Ceftazadime, Chloramphenicol, Ciprofloxacin, Doxycycline, Enrofloxacin, Gentamicin, Piperacillin, Ticarcillin, and Trimethoprim Sulfadimethazole. The antimicrobial sensitivity plates were incubated at 37° for 24 hours under aerobic conditions, then the distance surrounding the antimicrobials disks was measured using a metric ruler and recorded.

Sixty-nine Gram-negative oxidase negative Enterobacteriaceae isolates have been collected from wildlife (n=24) and the environment (n=45), and six coagulase positive *Staphylococcus* have been isolated from the environment (2 *S. intermedius*; 1 *S. aureus*) and wildlife (4 *S. intermedius*). To date, antimicrobial sensitivity testing has been performed on sixty-one organisms from the environment (n=37) and wildlife (n=24). Twenty-eight of the thirty-seven environmental samples had antimicrobial resistance patterns. Nineteen of the twenty-four wildlife samples had antimicrobial resistance patterns. Further testing will be performed through September of 2002.

The efficacy of Killed Equine Influenza Virus Vaccine Versus Attenuated Live Equine Virus Vaccine in Geriatric Horses. Darrel Dawsey, D. Dennis French, Marilyn Dietrich, David W. Horohov. Department of Pathobiological Sciences, Louisiana State University.

Equine influenza virus causes serious economic losses to the equine industry. While vaccination remains the main approach to prevent influenza infections, all horses are not equally protected following vaccination. Recently it has been documented that older horses do not respond as well as younger horses to a commercially available, inactivated equine influenza virus vaccine. The possibility that vaccination with an alternative form of the vaccine might prove more effective has been not been addressed. Here we examined the serological response of a population of old (≥ 15 years) horses following vaccination with either a killed virus vaccine or an attenuated live virus vaccine. A group of younger (≤ 15 years) of horses also received the attenuated live virus vaccine. We also assessed the in vitro immune response to the mitogen phytohemagglutinin (PHA) in a subpopulation of these horses. We observed an age-dependent antibody response to both vaccines. While the older horses responded poorly to the

inactivated virus vaccine when compared to the younger horses. An age-dependent response was also seen in the in vitro proliferative response with the older horses exhibiting a decreased response compared to the younger horses. This decreased proliferative response was not associated with an increase in the number of apoptotic cells in the cultures. Based on these results we conclude that the older horses exhibited a deficit in their immune response as measured both in vivo and in vitro. The increased systemic antibody response of the older horses to the live virus vaccine may have been the result of greater replication of the attenuated virus and thus more stimulation of a systemic immune response.

Antiviral Properties of Surfactant Associated Protein D in Bronchoalveolar Lavage Fluid of Young Foals. L. J. Gallegos, K.S. Bowles, S.S. Pourciau, R.E. Beadle, S.E. Mouch. Department of Pathobiological Sciences, Louisiana State University.

Growing evidence suggests that surfactant associated protein D (SP-D) is an important component of the innate immune system in the lungs of animals. Many studies have been done to characterize SP-D and its response to pathogens in humans, rats, mice and cattle, but little work has been done in horses. This study examines the role of SP-D in foals as a first defense against equine influenza virus (EIV). Whole bronchoalveolar lavage fluid (BALF) was extracted from pony foals of various ages. The antiviral activity of BALF and concentrated BALF was examined using a modification of a serum neutralization assay with Manin-Darby canine kidney cells grown to confluency on a 96 well plate. Antiviral activity was determined by the ability of the concentrated protein to protect the cells from lysis. The amount of cells protected was determined using 0.05% crystal violet stain and measuring the level of intensity of the stain with an ELISA plate reader. Initial results indicated that concentrated BALF had mild protective activity in three of five animals sampled. Future work will focus on the purification of SP-D protein using a maltose-agarose affinity column. The purified protein will then be used in the neutralization assay to determine if there is any increase in protection as compared to the initial results. Finally, RNA was extracted from fresh lung tissue of foals and adult horses and reverse transcribed to cDNA. From this cDNA we attempted to clone and sequence equine SP-D using degenerate PCR primers obtained from homologies of reported protein sequences on PubMed and use of the BlockMaker and CODEHOP program available on the Internet. Our initial results (from two PCR runs) did not produce any product. Attempts to clone SP-D will continue using several different primers and a PCR Optimizer Kit. Once SP-D is cloned it will then be expressed in COS cells and the recombinant protein tested for antiviral activity. The results will be compared with the previous SP-D protein results acquired from the BALFs. The conclusion of this study will help further characterize the developing immune system of foals through examining the role of SP-D in response to EIV.

The Ability of a Nitroglycerine Transdermal System to Deliver Nitric Oxide to the Equine Digital Vasculature. Meghan Gilhooly, S.C. Eades, R. Moore. Veterinary Clinical Sciences, Louisiana State University.

Equine laminitis is a debilitating, excruciatingly painful, and often career-ending or life-threatening disease. Although the pathogenesis of laminitis is still unknown, the current hypothesis suggests that vasoconstriction of the digital vessels arises from an imbalance of Starling forces and two endogenous vasoactive substances, nitric oxide (NO; decreased amounts leading to vasoconstriction) and endothelin-1 (ET-1; increased amounts leading to vasoconstriction) thereby causing laminar ischemic necrosis. NO has been identified as a potent vasodilator. Currently, nitroglycerine (GTN) patches are applied to the palmar digital vessels as a treatment of laminitis. The general hypothesis is that NO will diffuse transdermally from the patches into the blood and cause relaxation of vascular smooth muscle and subsequent vasodilation. This will reverse some of the ischemic effects by allowing reperfusion. The purpose of this study is to document that GTN patches are an effective means of administering NO to increase digital blood flow. Six adult healthy horses will have Doppler flow probes placed around their palmar digital arteries for the purpose of measuring flow. In addition, each horse will have its medial digital vein catheterized for the purpose of blood collection. After the surgery, GTN patches or GTN cream will be applied to the palmar digital vessels for 120 minutes. Blood flow and venous NO concentration will be measured for 60 minutes before, 120 minutes during, and 60 minutes after the patches have been applied. Results of this study will lead to a better understanding of the efficacy of this commonly used clinical treatment.

Determining the Seroprevalence of West Nile Virus in Chelonians from Louisiana

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The West Nile virus (WNV) was first isolated in the West Nile District of Uganda in 1937. In recent years, however, the WNV has emerged in temperate regions of North America presenting a threat to both human and animal health. The most serious manifestation of the virus is fatal encephalitis in humans, horses and certain domestic and wild bird species. The West Nile virus is a flavivirus that has an icosahedral nucleocapsid of approximately 40-60 nm and single stranded (+) RNA of approximately 10,000-11,000 bases. The virus has a complex life cycle involving a primary host, usually a bird, and a primary vector, the mosquito. Humans and domestic animals do not appear to act as primary hosts because they do not produce a significant viremia. However, to date, the research investigating the life cycle of the WNV in United States has been limited to surveillance work. Reptiles have been identified as hosts for WNV in eastern hemisphere, but to date have not been evaluated as potential host in the United States.

A cross sectional study is being performed to determine exposure and viremic status of reptiles in Louisiana. Chelonians are being collected from wild populations from those parishes where WNV positive horses and birds have been identified and from a reptile collector, who exports wild caught reptiles from Louisiana. To date, 21 chelonians have been evaluated, including 20 red-eared sliders (*Trachemys scripta elegans*) and one logger-head musk turtle (*Sternotheus mura*). The chelonians were collected from St. James parish. The chelonians were manually restrained for blood collection. A 22-gauge needle fastened to a 3ml syringe was used to collect 2 ml of blood from the supravertebral sinus. Blood was separated into an anticoagulant tube containing lithium heparin (~0.5ml) and a sterile clot tube (~1.5ml). The whole blood was centrifuged and the buffy coat removed. The white blood cells were added to 5:1 parts RNA later and frozen at 0°C until being processed for RT-PCR. The sterile clot tubes were centrifuged for 15 minutes at 10,000 rpm and the serum removed for HI testing. Hemagglutination Inhibition (HI) involves the combination of cultured WNV and serum from the collected blood sample. If antibodies are present in the serum they will attach to the virus. The virus-serum mixture is then incubated over night at 4°C. Avian blood (goose) is then added to the virus-serum mixture and allowed to incubate for one hour. If there are antibodies in the serum they will bind the virus and this will inhibit the agglutination of the blood cells. If no antibodies are present then the virus it will agglutinate the blood cells. HI allows for the determination of exposure to the virus. In Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) white blood cells are lysed and viral RNA is extracted. The viral RNA, if present, is combined with RNA primers for WNV and with RNA transcriptase and placed into a thermal-cycler in order to convert the RNA into cDNA and amplify it. The amplified sample is then resolved by gel electrophoresis using a 2% agarose gel. The samples are run with a positive control to determine the presence of virus in the blood sample. The 21 chelonian samples, which have already been evaluated with HI, were all negative. RT-PCR results and further samples for HI are pending.

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Characterizing Aerobic Bacteria and Antibiotic Resistance in Shark Populations. M-C Holley, J. K. Blackburn, M. A. Mitchell, B.A. Thompson. Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, La 70803.

Antibiotic resistant (ABR) microbes are a potential source of infectious disease for humans and animals. Resistant strains of microbes complicate the treatment of infectious disease and require new and more expensive antibiotics to replace those that have been used to treat infection in the past. In some cases, there are no available drugs for treatment. Recent research has found ABR in apex predators from terrestrial environments (Mitchell et.al. 2001). However, no published data exist for ABR in marine fishes. This research used sharks as sentinels for ABR because they are complex apex predators that use marine environments. The goals of this study were to characterize the aerobic microorganisms from the cloaca of sharks and the ABR patterns of these organisms. This knowledge is of great interest to human and animal health specialists. Based on the results of studies incorporating terrestrial apex predators, it was hypothesized that resistance would be found in at least 5% of the organisms identified.

A total of 25 wild caught sharks were recruited for this study, including 12 bull sharks, *Carcharhinus leucas*, from Louisiana, four nurse sharks, *Ginglymostoma cirratum*, from the Florida Keys, and nine nurse sharks from Belize. Cloacal swabs were collected from all sharks using sterile culettes and refrigerated or kept on ice until they could be processed. Blood (BA) and MacConkey agars and BHI enrichment broth were used initially to isolate microorganisms at 37C for 24 hours and then transferred to Mueller-Hinton agar plates. Antimicrobial diffusion discs, representing 12 antibiotics, were placed on the plates and zones of inhibition were measured following 24 hours of incubation.

Fifty-two organisms were isolated from the three study sites. Twenty-two organisms were isolated from the Louisiana bull sharks, 18 from the Florida nurse sharks, and 12 from the Belize nurse sharks. The organisms were identified to the generic level and represented 12 genera. Fifteen (29%) of the 52 organisms were not characterized to the generic level using the chosen methods, but were characterized by their Gram stain and biochemical characteristics and therefore included in the antimicrobial resistance surveillance.

Resistance was found in all three geographic locations and for both species of sharks. Additionally, resistance was found for each non-synthetic and synthetic drug tested in at least a portion of the isolates. The areas studied included those with large human interaction, such as the Florida Keys study site. That resistance was found in all three locations, for both species, and for every drug tested, goes beyond the expectations of the original hypothesis. Greater exploration of additional species, habitats, and reasons for such resistant strains of microbes is necessary. This information provides the first examination of ABR in an apex predator in a marine environment and suggests that epidemiologic investigation to identify the point sources and causes for these ABR patterns should be performed.

In Vitro Effects of Feline Reproductive Hormones on Replication of *Bartonella henselae*. K. A. Parr and K.L. O'Reilly, Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803

The domestic cat is the carrier of *Bartonella henselae* which causes cat-scratch disease (CSD) in immunocompetent humans and more severe diseases in immunocompromized individuals, including life-threatening conditions such as bacillary peliosis, bacillary angiomatosis, and recurrent bacteremia. The infection in cats is characterized by bacteremia of 6 to 12 weeks duration that may recur at a later time. Cats develop a high level of antibody in response to infection, but the mechanism of control is not known. In previous experiments in our laboratory, 11 cats out of 40 had recurrent bacteremia, of the 11 that recrudesced, 9 were female and 2 were male. Of the females, 7/9 recrudesced during a heat cycle and 2/9 recrudesced at parturition. These results suggest that recurrent bacteremia may be associated with fluctuations in reproductive hormones. In particular, it may be signaled by high-levels of estradiol, low levels of progesterone, or a change in the ratio of these two hormones, as these are the conditions present at both estrus and parturition. To investigate this subject, we examined the effect of exogenous hormones growth and survival of *B. henselae* in bacterial medium and in feline macrophage cultures. No effect on *B. henselae* growth was seen to the addition of progesterone, dihydrotestosterone or 17- β -estradiol to bacterial medium. An inverse correlation was observed between progesterone dose and bacterial survival in macrophage cultures while a direct correlation was seen between the addition of 17- β -estradiol and bacterial survival in macrophage cultures. This pilot study suggests that hormone levels influence *B. henselae* replication and additional, more detailed studies are ongoing.

Targeting Angiogenesis With Tum5: Killing Cancer By Starving Tumors?

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Tumstatin is the NC1 (noncollagenous) domain of the $\alpha 3$ subunit of mammalian type IV collagen (also known as Goodpasture's antigen), a major macro-molecular component of basement membranes. It has been reported that a fragment of tumstatin, designated Tum5, is responsible for the anti-angiogenic properties of tumstatin observed *in vitro*. Angiogenesis, growth of new blood vessels from existing vasculature, is a key target for cancer treatment because it serves a central role in tumor growth and metastasis.

We designed polymerase chain reaction (PCR) primers from the published sequence of canine $\alpha 3$ subunit from type IV collagen and amplified the putative coding region for canine Tum5 from canine kidney RNA. The coding sequence was inserted into a prokaryotic expression vector, pBAD-TOPO, and recombinant Tum5 was produced in *Escherichia coli*. The recombinant product was purified by affinity and size exclusion chromatography.

Purified recombinant Tum5 was used to treat bovine pulmonary arterial endothelial cells and canine oral melanoma cells. It was determined that Tum5 exhibited a strong dose dependent relationship on the viability of pulmonary arterial cells while having no effects on the melanoma cells. Additionally apoptotic assays indicated that the recombinant Tum5 exhibited its cellular effects through apoptotic pathways.

In Vitro Characterization of Bronchial Response to Neurokinin-A in Clinically Healthy Horses and Those with Obstructive Pulmonary Disease. Shawn Wilson, R.M. Moore, E. Dequeant and C. S. Venugopal. Veterinary Clinical Sciences, Louisiana State University.

Summer pasture-associated obstructive pulmonary disease (SPAOPD) is a common respiratory disease of horses in the Southern region of the United States. It is characterized by airway obstruction, hyperreactivity with increased mucus secretion and severe respiratory distress. Several mediators have been shown to cause constriction of the bronchial smooth muscles thus leading to increased resistance in the airways. One such mediator that has received attention in recent years is Neurokinin-A (NK-A). NK-A is a tachykinin that has been shown to cause bronchial constriction in asthmatic humans and guinea pigs. The role of this mediator has not been elucidated in equines as of yet. The purpose of this study is to examine if NK-A will cause contractions in clinically healthy equine bronchial tissue and then to compare those results to that of horses with SPAOPD.

Horses that are destined for euthanasia are divided into two groups via clinical scoring into clinically healthy or SPAOPD-affected. The affected group is confirmed by pulmonary function testing the day before the euthanasia is scheduled. A section of the right diaphragmatic lobe of lung is removed from euthanatized horses. Bronchial rings are made 4mm wide at about the 4th to 7th generation. The rings are fixed to organ baths at 37°C with oxygenated Tyrode's solution and connected to force transducers interfaced with a polygraph for contractile response measurements. A 2g initial tension is applied to each ring before they are allowed to equilibrate for a period of 45 minutes and is followed by a cumulative concentration-response relationship determination for NK-A (10⁻⁸ to 10^{-4.5}M). Tissues are divided into four groups: epithelium intact, epithelium denuded, epithelium intact NANC (Non-adrenergic, non-cholinergic), epithelium denuded NANC. The same protocol is repeated on tissues treated with a NK-A antagonist. The responses are statistically analyzed using ANOVA and if needed by post-hoc analysis using Tukey's test.

Preliminary studies were completed using five commercially available tachykinins: Neurokinin A/Substance K, Neurokinin A (4-10), [Ala5, Beta Ala8] Neurokinin A (4-10), (Beta Ala8) Neurokinin A (4-10), and Substance P. From these studies only Neurokinin A (4-10) has shown a contractile response in both clinically healthy tissues. Therefore, the remainder of the study will focus on Neurokinin A (4-10).