# 2015 Research Forum

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VS = Veterinary Student, HO = House Officer, GS = Graduate Student  
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VS = Veterinary Student, HO = House Officer, GS = Graduate Student
NS = Non Student, UG = Undergraduate Student
INFLAMMATORY CYTOKINE PROFILES IN CATS WITH DEGENERATIVE JOINT DISEASE (DJD)-ASSOCIATED PAIN

Lauren Aldrich, DVM Student
Duncan Lascelles, BSc, BVSc, PhD, CertVA, DSAS(ST), DECVS, DACVS
& Margaret Gruen*, DVM, DACVB, MVPH
laaldric@ncsu.edu; megruen@ncsu.edu
Affiliations: NCSU CVM Comparative Pain Research Laboratory

*corresponding author

ABSTRACT

OBJECTIVES: There are no analgesics approved in the U.S. for long-term use in cats with painful DJD, due partly to the challenge of measuring pain. Serum biomarkers might have utility as objective measures of pain in cats. The purpose of this project was to compare cytokine profiles of DJD-affected cats with varying pain and chronic kidney disease (CKD) burdens to identify pain biomarkers. Our central hypothesis was that cytokine profiles of cats with DJD-associated pain differ from those of normal cats, and can be used as measures of pain in cats with DJD. Further, we expected inflammatory burden to be greater in cats with DJD and CKD than in cats with DJD alone. In this study, serum levels of 19 cytokines were measured and compared in 186 well-phenotyped cats with and without DJD-pain/CKD.

METHODS: Multiplex bead-based immunoassays were used to measure cytokine levels in serum from cats phenotyped for pain score, radiographic DJD score and CKD status. Data will be evaluated using analysis of variance, and biomarker patterns will also be investigated using correlations and principal components analysis (PCA).

EXPECTED RESULTS: Based on our pilot study results and the human literature, we expect that IL-1β, IL-6, IL-4, TNFα, and MCP-1 will correlate with pain score. We expect overall inflammatory cytokine levels to be higher in cats with CKD.

CONCLUSIONS & RELEVANCE: If biomarkers of DJD-related pain are identified, they may provide potential objective measures of pain phenotype. Additionally, the results may generate hypotheses regarding therapeutic targets for DJD-associated pain.

Funding source: Winn Feline Foundation
ANGIOPELLOSIS: A NEW MECHANISM FOR CELL EXTRAVASATION

Author: Tyler Allen (Graduate Student)
Co-Authors: Maliha Talib, David Gracieux, Jeffrey Yoder, Ke Cheng
Email: taallen@ncsu.edu

Affiliations: 1. Department of Molecular Biomedical Sciences and Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University;
2. Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University

Background
Stem cell therapy is a promising strategy of regenerative medicine. Intravenous injection has been proven as safe and effective methods for cell delivery. For stem cells to exert their therapeutic effect, they must cross the blood vessel wall and enter the surrounding tissues. However, the mechanism of this phenomenon has yet to be characterized. Using intra-vital microscopy and a transgenic zebrafish model, we discovered a previously unreported novel mechanism of stem cell extravasation, which we have termed Angio-pello-sis.

Methods
The Fli1a:EGFP zebrafish strain allows for unique visualization of blood vessels during the development, as they produce the green fluorescent protein (gfp) exclusively in the vasculature. Multiple cell types were used, including leukocytes, cardiac stem cells, and tumor cells. These cells were labelled with a fluorescent dye, and injected directly into the blood stream of the embryos at 48 hours post fertilization and then imaged to capture the mechanism of extravasation.

Results
The cardiac stem cells, once injected, underwent a distinct method of extravasation that was markedly different from that of other cells found in the blood stream, such as leukocytes. In this mechanism, the vascular wall undergoes an extensive remodeling to allow the cell to exit the lumen, while the cell itself remains distinctively passive in activity (see Figure 1). We term this new cell extravasation process as Angio-pello-sis (Angio = related to blood vessels; pello = expel).

Conclusions
Angiopellosis represents an alternative mechanism of cell extravasation to the prevailing theory of diapedesis. A better understanding of this mechanism will lead to improved methods for stem cell therapies, and conversely, better strategies to mitigate cancer metastasis.
IDENTIFICATION OF NOVEL GENES AFFECTING LEFT-RIGHT MORPHOGENESIS IN THE EMBRYONIC HEART

Martha Alonzo-Johnsen, Postdoctoral Research Scholar
Nanette Nascone-Yoder
mralonzo@ncsu.edu, nmnascon@ncsu.edu
NCSU CVM MBS

The developing heart undergoes a symmetry-breaking event when it changes from a linear heart tube to a right looped structure. This looping process is crucial for correctly positioning the cardiac chambers and associated vascular structures within the circulatory system. Previous studies have shown that molecular markers such as transcription factor Pitx2c are asymmetrically expressed in the heart tube and are required for proper heart looping. However, the downstream cellular and molecular mechanisms that lead to this asymmetry remain elusive. The objective of this study is to identify differentially expressed transcripts between the contralateral halves of the heart tube to discover new molecules that control left-right asymmetric morphogenesis. To accomplish this objective, we used the unique embryological features of a novel model amphibian, *Lepidobatrachus laevis*. *Lepidobatrachus* has massive embryos that facilitate precise excision of the left and right halves of the early heart, which we have used for RNA-sequencing. Upon analyzing the data, we have prioritized a list of twenty candidate genes whose expression is either left- or right-enriched. The goal of this project is to validate the biological relevance of these left- or right-enriched transcripts for asymmetric heart morphogenesis.
A ROLE FOR THE DOWN’S SYNDROME-ASSOCIATED GENE SIM2 IN PROPER LEFT-RIGHT ORGAN ASYMMETRY

Nirav M. Amin (namin@ncsu.edu)
Nanette Nascone-Yoder (nmnascon@ncsu.edu)

Department of Molecular Biomedical Sciences
College of Veterinary Medicine, North Carolina State University

Structural birth defects are a leading cause of infant mortality in the United States. Many of these defects are associated with abnormal anatomical left-right asymmetry. Despite the importance of organizing internal organ systems along the left-right (L-R) axis, very little is known about the molecular events that control this process. In an effort to elucidate the genetic mechanisms that shape the L-R asymmetry of organs within the developing embryo, we hypothesized that there are groups of genes which are expressed in an asymmetric expression pattern during development. To test this hypothesis, we have taken advantage of the exceptionally large Budgett’s frog embryo in order to profile gene expression by RNA-seq in the left versus right halves of the developing stomach. In addition to identifying known genes in L-R organ development, our analyses of these data have identified a handful of genes not previously described to be involved in L-R asymmetry. At least one gene, Singleminded2 (Sim2), is expressed downstream of, and is necessary for, the function of the transcription factor Pitx2, a key determinant of L-R asymmetry in all vertebrates. Interestingly, Sim2 has been associated with Down’s syndrome, which has been associated with congenital anomalies of the gastrointestinal tract. These results show that levels of Sim2 must be tightly regulated to lead to proper L-R asymmetry. Furthermore, these studies will help elucidate the mechanisms by which the L-R asymmetric development of internal organs occurs and provide additional candidates for study in individuals affected by laterality-related birth defects.

This work has been funded by NIH (RO1 DK085300, R21 OD017963) awards to N.N-Y.
D-XYLOSE ASSAY IDENTIFIES INTESTINAL MALABSORPTION IN KITTENS EXPERIMENTALLY INFECTED WITH ENTEROPATHOGENIC E. COLI

Sophia S. Amirsultan (Veterinary student)
Victoria E. Watson, Stephen H. Stauffer, Megan E. Jacob and Jody L. Gookin
Email:ssamirsu@ncsu.edu,vewatson@ncsu.edu,shstauff@ncsu.edu,megan_jacob@ncsu.edu, jody_gookin@ncsu.edu
NCSU CVM, Raleigh NC

Diarrheal disease is a leading cause of death in foster-age kittens. In prior studies, we observed enteropathogenic E.coli (EPEC) attaching to the intestinal epithelium of kittens that died in foster care. Pathogenic effects of EPEC derive from their ability to attach to intestinal epithelium, efface microvilli, and impair intestinal absorption. The goal of these studies was to non-invasively measure the effect of EPEC on intestinal absorption in experimentally infected kittens. D-xylose, a pentose monosaccharide, is absorbed from the GI tract into systemic circulation unchanged and therefore is an optimal marker for evaluation of small intestinal absorption. D-xylose was administered by gavage to eight kittens before and after infection with EPEC, and its recovery into circulation was measured using a colorimetric assay. The sensitivity and reproducibility of the D-xylose assay was validated using normal cat serum spiked with increasing concentrations of D-xylose or glucose to create a standard curve. For analysis of data from EPEC infected kittens, pre-infection D-xylose absorption was used as baseline for the post-infection absorption and compared using a paired Student’s t-test. Our results showed a statistically significant decrease in serum D-xylose concentration following infection of kittens with EPEC, implying that EPEC decreases intestinal absorption in infected kittens. The assay was linear within the test range (3.25-100 mg/dL) of serum D-xylose. No significant interference from serum proteins or serum glucose (30-300 mg/dl) was observed. With further refinement, this assay could serve as an important diagnostic tool in the detection of feline intestinal malabsorption in a clinical setting.

Research Grants: NCSU Research Innovation and Seed Fund and College of Veterinary Medicine State Appropriated Research Funds
Student Support: NIH/T35 Interdisciplinary Biomedical Research Program, Grant Number-NIH T35OD011070.
DEVELOPMENT AND VALIDATION OF A NEW DIAGNOSTIC qPCR ASSAY FOR DETECTION OF BABESIA SPECIES

Nikole R Archer, pre-veterinary student

Co-Authors: Barbara A. Qurollo, Barbara C. Hegarty, Julie Bradley, Susan J. Tornquist, Kathryn. G. Schlaich, Edward B. Breitschwerdt

Email addresses: jdtyrrel@ncsu.edu, baqurollo@ncsu.edu, bhegarty@ncsu.edu, julie_brady@ncsu.edu, susan.tornquist@oregonstate.edu, schlaick@onid.oregonstate.edu, ebbreits@ncsu.edu

Affiliations: North Carolina State University, College of Veterinary Medicine, Raleigh, NC (J.D. Tyrrell, B.A. Qurollo, B.C. Hegarty, J. Bradley, E.B. Breitschwerdt)

Abstract: Babesia species are tick-transmitted pathogens that infect erythrocytes of several mammalian hosts, including dogs, cats, horses, cows, humans and other wildlife. The Vector-borne disease diagnostic laboratory (VBDDL) developed a sensitive and specific quantitative PCR assay targeting the LSU 5-4 genes located on the multi-copied mitochondrial genome found in Babesia spp. This assay detects a broader range of Babesia spp. in diagnostic samples. The assay's limit of detection was 5 copies/reaction, and it did not amplify nonspecific DNA. Compared with the 18S rRNA gene PCR target, the mitochondrial LSU 5-4 target offered an improved molecular diagnostic assay to detect Babesia spp.

Funding: VBBDL-NCSU
Endangered sea turtles are at increasing risk of oil exposure from off-shore drilling and increased maritime oil transport. Despite this, little is known of the effects of crude oil on sea turtles. We used proton-nuclear magnetic resonance spectroscopy (1H-NMR)-based metabolomics to examine the hypothesis that oil exposure would perturb the metabolome of hatchling loggerhead sea turtles (Caretta caretta) in whole blood and skeletal muscle, tissues suitable for vital field sampling. We predicted alterations in the Tricarboxcylic Acid Cycle, Cori Cycle (lactic acid), and other energetic pathways for both tissues. Samples were collected from 11 animals subjected to 96-hour cutaneous exposure to sea water (control, n=6) or crude oil (exposed, n=5) beginning 3-days post-hatching. Tissues were flash-frozen and later processed with a cell lysis reagent (blood) or extraction with 8:4:3 v:v:v chloroform, methanol, and water (muscle). Changes in eight metabolites readily identified in whole blood indicated depletion of glucose and 3-hydroxybutyrate and accumulation of myo-inositol, creatinine, glutamate, and lactate with oil exposure. Changes in twelve readily identified metabolites in skeletal muscle showed depletion of glutamine, glycine, choline, and alanine associated with oil exposure. This work provides important baseline information for hatchling sea turtle health assessment and supports the hypothesis of perturbation of key energetic pathways in hatchlings exposed to crude oil. Future studies will use 2-D experiments to identify additional metabolites in these and other tissues of oil-exposed hatchlings and explore alternative data analysis to improve interpretation of 1H-NMR metabolomic data from small biopsy samples.

Research supported by: Oiled Wildlife Care Network
Fear-conditioned analgesia (FCA) is a survival response defined as a reduction in nociceptive behaviors upon re-exposure to a conditioned aversive stimulus. FCA is facilitated through activation of the descending inhibitory pain pathway which originates in brain regions such as the amygdala. In the central nucleus of the amygdala (CEA), it is unclear how the local (enkephalin-positive [ENK]) and the projection (corticotropin-releasing factor-positive [CRF]) neurons interact to mediate FCA. We hypothesize that conditioned fear expression induces activation of both ENK and CRF neurons of the CEA and that FCA activates CRF neurons to a greater degree than either conditioned fear or pain alone.

The behavior paradigm consisted of Pavlovian fear conditioning (FC; re-exposure to an arena associated with footshock) combined with the formalin test of persistent pain resulting in four groups: control (no FC-saline), conditioned fear (FC-saline), pain (no FC-formalin), and FCA (FC-formalin). After the behavior tests, brains were fixed, sliced, and immunostained for ENK, CRF, and a protein marker of neuronal activation. The different roles of the phenotypic populations were determined by calculating the percentages of activated ENK and CRF between the four groups.

Early results showed a higher percentage of activated CRF neurons in the CEA in the FCA group compared to conditioned fear and pain alone. This suggests that a CRF population of neurons is important in an amygdala-mediated, analgesic response to a conditioned aversive stimulus. Furthermore, CRF neurons of the CEA could be a target for future pain therapeutics.

Funding source: T35 Interdisciplinary Biomedical Research Training Program (IBRTP). NIH grant- T35OD011070
Mesenchymal stromal cells (MSCs) are a promising cell source for musculoskeletal therapies. Allogeneic MSCs can be immunogenic, however, due to recipient recognition of mismatched Major Histocompatibility Complex (MHC) molecules. Transforming growth factor-β2 (TGF-β2) is an immunomodulatory cytokine capable of suppressing surface expression of MHC-I and II. The purpose of this study was to determine if treating equine MSCs with recombinant human TGF-β2 decreases surface expression of MHC-I and II. We compared MSCs continuously cultured with 1, 5, or 10 ng/ml TGF-β2 with untreated MSCs. Monoclonal antibodies and fluorescence activated cell sorting (FACS) were used to detect surface expression of MHC molecules after culturing MSCs for two passages. MSCs treated with 1 ng/ml TGF-β2 had 3.5 fold decrease from control in MHC-I GMFI, 5 ng/ml a 3.7 fold decrease, and 10 ng/ml a 3.1 fold decrease. MHC-II expression in two positive animals was also significantly reduced compared to untreated MSCs. The Dako QIFIKIT® assay was used to quantify MHC-I surface expression confirming that TGF-β2 treated MSCs have significantly fewer MHC-I surface molecules than untreated MSCs and similar expression levels to equine fetal fibroblasts, which are non-immunogenic. To determine if TGF-β2 affects equine MSC trilineage potential, we induced treated and untreated MSCs to undergo adipogenesis, osteogenesis, and chondrogenesis. Robust trilineage differentiation equivalent to untreated MSCs was found only for the 1 ng/ml TGF-β2 group. These results revealed that TGF-β2 significantly decreases MHC-I and II surface expression on equine MSCs and that treatment with continuous 1 ng/ml TGF-β2 does not affect multipotency.
QUANTIFYING CENTER OF PRESSURE VARIABILITY IN CHONDRODYSTROPHOID DOGS

Author: Sarah Blau, veterinary student
Co-Authors: Leighanne Davis, William Pfitzner, Angela Gorney, Carly Dohse, Kimberly Williams, Ji-Hey Lim, Natasha Olby, Greg Sawicki
Email: srblau@ncsu.edu
Affiliations: NCSU CVM and UNC/NCSU Biomedical Engineering

Abstract: Chondrodystrophoid breeds have a high incidence of acute spinal cord injury due to disc herniations. Analysis of center of pressure (COP) variability of these dogs as they recover from spinal injury may provide a way to document recovery and to compare outcomes in clinical trials. The purpose of this descriptive study was to quantify normal range of COP variability in healthy chondrodystrophoid dogs. Fifteen chondrodystrophoid dogs were assessed individually walking on a force-plate treadmill that recorded the location of the dogs’ COP. A minimum of nine trials was recorded for each dog over the course of one or more different-day sessions. The root mean squared (RMS) values for changes in COP location in the longitudinal (y) and horizontal (x) directions were calculated to determine the range of COP variability. Of the 15 dogs tested, three would not walk consistently on the treadmill, and one dog’s data was not appropriately captured by the force plate. Data from 206 trials from the remaining 11 dogs was processed and analyzed. COP variability RMS values ranged from 0.0046 to 0.0285 (median: 0.0138) in the x direction and 0.0085 to 0.0555 (median: 0.0177) in the y direction. Repeat measurements in six dogs on different-day sessions suggest little variation; however, further statistical analysis is needed to determine variability between sessions. We conclude that COP variability can be measured in walking dogs. Chondrodystrophoid dogs recovering from spinal cord injuries will be evaluated in future research to determine whether COP variability is a reliable measure of paraparesis.

Funding Sources: NIH/ORIP T35ODO11070, NCSU Veterinary Scholars Program, NCSU, Merial Inc., Department of Defense, Morris Animal Foundation, and NCSU CVM Center for Comparative Medicine and Translational Research
LOSS OF C/EBPβ INCREASES THE PROAPOPTOTIC FUNCTION OF p53 BY ENHANCING EXPRESSION OF p53 TARGET GENES.

Lauren E. Brierley¹ (veterinary student)  
Jonathan R. Hall², Hann W. Tam², Keith E. Linder¹, Robert C. Smart²

lebrierl@ncsu.edu, jrhall@ncsu.edu, turhiril@gmail.com, kelinder@ncsu.edu, rcsmart@ncsu.edu

¹ College of Veterinary Medicine, North Carolina State University  
² Department of Biological Sciences, North Carolina State University

Abstract:

p53 is a transcription factor that plays a critical role in the regulation of apoptosis, cell cycle arrest, and senescence. Disruption in p53 function is a common event in cancer. C/EBPβ is a basic leucine zipper transcription factor, which has been implicated in inhibiting p53 apoptotic function. Genetic ablation of C/EBPβ results in increased levels of p53 and increased apoptosis in response to DNA damage induced by chemical carcinogens, UVB radiation, and chemotherapeutic agents. Understanding how the loss of C/EBPβ results in increased p53-mediated apoptosis could have important implications for future molecular cancer therapies aimed at increasing apoptosis in p53 proficient tumors. In this study, we aimed to learn more about how the loss of C/EBPβ increases pro-apoptotic function of p53 and whether the anti-proliferation function of p53 is also enhanced in C/EBPβ deficient cells. To this end, we examined the expression of pro-apoptotic, anti-apoptotic, and anti-proliferative p53 target genes at both the transcript and protein levels in UVB-treated control keratinocytes and C/EBPβ depleted keratinocytes. Preliminary results from immunoblot staining showed that a loss of C/EBPβ resulted in higher levels of anti-proliferative C/EBPβ compared to control cells as predicted. C/EBPβ decreased 6hrs after UVB exposure in C/EBPβ depleted cells. p21 (anti-proliferative) and BAX (apoptotic) were higher in untreated than UVB treated cells, suggesting that p21 and BAX are not dependent on C/EBPβ. Additional results will be presented.

Funding Sources:
NIH T35 Interdisciplinary Biomedical Research Grant T35OD011070  
Center for Human Health and the Environment
ADAPTATION OF A RODENT BURIED FOOD PELLET TEST FOR EVALUATION OF OLFACTION IN DOMESTICATED CATS

Heather C. Brown¹, veterinary student
Melanie L. Foster¹, David C. Dorman¹
hcbrown3@ncsu.edu, melanie_foster@ncsu.edu, david_dorman@ncsu.edu
¹North Carolina State University College of Veterinary Medicine

The buried food pellet test is widely used in toxicology to assess a rodent’s ability to smell food-related odors. The primary purpose of this study was to adapt this test for cats. Young (≤ 7 years old) and aged (≥ 11 years old) cats were used. We hypothesize that aged cats experience deficits in olfactory ability. Cats were habituated to a 0.73 m³ cage, taught to find treats, and tested. Testing involved two 10-minute buried food trials using one pound of autoclaved shredded paper. Cats were not tested if they exhibited stress (e.g., excessive vocalization, escape behaviors) or refused to eat treats. 8/16 cats failed to habituate to the cage; however, the majority of cats that successfully habituated located the food treat within 1 minute. No statistically significant difference was seen between young (n=5) and old (n=3) cats (p=0.82). The same treats and paper or corncob bedding were used to conduct buried food pellet tests in adult rats to assess the impact of substrate and experience on test performance. The initial trial with paper was associated with a significantly longer time than the subsequent two trials with male rats (p<0.0001) and for the females compared to their first (p=0.0128) and third (p=0.0043) sessions (bedding). This diminished performance is likely due to the shredded paper acting as a novel substrate and distractor. Habituation and substrate optimization may be needed in order for the buried food pellet test to be used to assess olfaction in cats.

Research Grant: Office of Naval Research, NCSU CVM Dorman Laboratory
It has been observed that absorbable sutures persist in cold-water fishes and amphibians longer than in mammals. We hypothesized that suture loops incubated at 4 and 25°C would hydrolyze more slowly than loops incubated at 37°C. Suture loops (n=6) formed using two absorbable monofilament 3-0 suture materials, poliglecaprone (Monocryl) and polyglyconate (Maxon), were incubated in filtered city water for 2, 4, 6, and 8 weeks at 4°C, 25°C, and 37°C. The maximum tensile load was measured as each loop was distracted to failure. There were no differences in failure loads for Maxon at 4°C over time. At 25°C, loop strength was increased to approximately 50N for 2, 4, 6 and 8 weeks compared to 28.5N at 0 weeks. At 37°C, the 2 and 4 week loop strength was greater than 0 weeks. The loop strength decreased at 6 (30N) and 8 weeks (15N). For Monocryl at 4°C, the 2, 4 and 8 week loops were stronger than the 0 week loops. There was a wide range of values for the 6 week loops. At 25°C, the 2 week loops (41N) were stronger than the 0 week loops (16.5N). Loop strength declined with time, with a failure load of 12.5N by 8 weeks. At 37°C, 2 week loops (25.5N) were stronger than 0 week loops (16.5N). Only 4 loops could be tested after 4 weeks (2N), and none after 6 or 8 weeks. Our findings confirm that sutures designed to be absorbable in warm-blooded mammals will weaken more slowly in poikilotherms.
EFFECT OF HMAG2 DEFICIENCY ON FETAL SURVIVAL AND PLACENTAL FUNCTION IN PIGS

Jaewook Chung (Research Assistant)1,2

Xia Zhang2, Bruce Colins2, Kayla Howard4, Charles Salmon4, Renan Sper2, Sean Simpson2, Sehwon Koh1,2, Jeffrey Sommer3, Clay Byrd4, William L. Flowers3, Robert M. Petters2,3 and Jorge A. Piedrahita1,2

japiedra@ncsu.edu

1Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC, USA. 2Functional Genomics, North Carolina State University, Raleigh, NC, USA. 3Department of Animal Science, North Carolina State University, Raleigh, NC, USA. 4Swine Educational Unit, Raleigh, NC, USA.

The high mobility group AT-hook 2 (HMGA2) proteins are involved in growth regulation by affecting cell proliferation and apoptosis in mammals. Mutations in this gene alter body size in mice and humans. In mice, compared to wild type littermates, adult homozygous mutants are 60% smaller whereas adult heterozygous mutants are 20% smaller. In order to determine whether a similar effect was seen in pigs a transgenic HMGA2 pig line was generated by gene targeting in fetal fibroblasts. An insertional mutation was performed to generate mutant cell lines by disrupting one allele of the HMGA2 gene. Using the mutant cell lines, female (n=6) and male (n=8) HMGA2+/- clones were generated by somatic cell nuclear transfer (SCNT). As in mice, adult mutant body weights and lengths resulted in approximately 75% in weight (p<0.0001) and 86% in length (p<0.0001) over control animals. Natural breeding of HMGA2+/- animals failed to produce HMGA2-/- piglets in any of four litters. We examined pregnancies at D40 and D78 gestations and were able to detect healthy HMGA2+/- fetuses at the expected Mendelian frequency. While the placentas from HMGA2+/- fetuses were normal at D40, HMGA2-/- placentas differed from HMGA2+/+ and HMGA2+/- placentas at D78. D78 HMGA2-/- placentas had reduced placental areolae and appeared necrotic. Histological measurements of villi showed reduced placental surface area in HMGA2-/- fetuses compared to HMGA2+/+. Our results suggest that the abnormal arrangement between endometrium and the HMGA2-/- chorionic membranes, may lead to late-gestation lethality of HMGA2-/- piglets due to poor maternal-fetal physiological exchange.

Funding Source: NIH Grant R21 OD010553.
ULTRASONIC ASSESSMENT OF BLADDER VOIDING IN NORMAL AND PARAPARETIC DOGS

Carly Dohse: Veterinary Student
Co-Authors: Natasha Olby, Angela Gorney, Sarah Blau, Ji-Hey Lim, Kim Williams, Gabriela Sieler
csstephe@ncsu.edu
NCSU CVM

Chondrodystrophoid breeds such as dachshunds have a high incidence of acute thoracolumbar spinal cord injury causing paraplegia and paralysis of the bladder. Quantitation of bladder emptying during recovery from thoracolumbar spinal cord injury could provide documentation of neurologic recovery. We hypothesized that recovery of voiding will correlate to recovery of hind limb motor function following spinal cord injury. The aims of this study were to determine efficiency of bladder voiding in normal dogs, describe changes in bladder voiding efficiency following spinal cord injury and correlate these changes to motor recovery.

The maximum length, width and depth of bladders of normal and paraparetic dogs were measured ultrasonographically. Measurements were repeated immediately after urination or manual expression. The volume was calculated using a validated formula (V= L x W x ((DL + DT)/2) x .625) (Atalan et al., 1998).

Fourteen normal and 13 neurologic dachshunds were measured 4-6 hours post urination and immediately after urinating. Median emptying was 92% (79.0-99.5%) of bladder volume in normal dogs (15 observations); ambulatory paraparetic dogs (11 observations from 6 dogs) emptied 92% of their volume (69.5-97.6%); 9 non-ambulatory paraparetic dogs (18 observations) emptied 87% of their volume (23.0-97.0%); 5 paraplegic dogs emptied 0%.

We conclude that bladder emptying can be quantified using ultrasonography and is a useful measure of neurologic recovery in dogs with spinal cord injury. Additional normal and neurologic dogs will be assessed to allow statistical examination of the correlation between gait grade and bladder emptying.

Funding: Merial, College of Veterinary Medicine, Veterinary Medical Foundation Fund for Discovery, CCMTR, Department of Defense, Morris Animal Foundation
IN VITRO ANALYSIS OF *LISTERIA MONOCYTOGENES* ATTENUATED MUTANTS FOR USE AS VACCINE VECTORS

Robert Dugger: Veterinary Student, Patricia A. Spears, Edward A. Havell

Paul Orndorff

radugger@ncsu.edu, paul_orndorff@ncsu.edu

Department of Population Health and Pathobiology, NCSU CVM

*Listeria monocytogenes* is most commonly known as a potential food borne bacterial pathogen. Due to Listeria’s distinct intracellular lifecycle, ease of genetic manipulation, and ability to stimulate cell mediated immunity, this gram positive bacterium has been extensively modified to express a variety of foreign antigens for vaccine and immunotherapeutic purposes. However, because of its inherent virulence, live attenuated listerial vaccine mutants are required for safety reasons. Striking a balance between safety while retaining immunostimulatory efficacy, through genetic manipulation, has not yet been achieved. The goal of my project is to establish the intracellular growth rates of four attenuated mutants that are candidates for development as live, orally-deliverable, vaccine and immunotherapeutic agents. Intracellular growth curves were obtained by mixing a mutant with a genetically marked parental reference strain and performing gentamicin protection assays using cultured murine enterocyte monolayers. Preliminary data indicates that one of the four mutant vaccine candidates, has a growth curve similar to that of the parent. The remaining three do not grow as efficiently as the parental. Ongoing work is directed at replicating and extending the present results. These results will be used as part of a down-selection process to identify a safe and efficacious live oral listerial vaccine platform for the expression of foreign antigens.

Research Support: Herbert Benjamin Prof. Endowment, NIH Grant Number: R21AI103549
PILOT EVALUATION OF A NOVEL UNILATERAL DECLAW MODEL AND EFFICACY OF AN EXTENDED RELEASE BUPRENORPHINE PRODUCT

Masataka Enomoto a Research Assistant
Patricia D. Kigin b, David Bledsoe b, Jon Hash a, Charles E. Smith c, and B. Duncan X. Lascelles a,d,e*

menomot@ncsu.edu; PKigin@central.com; daviddrdad@gmail.com; sureshotxx@yahoo.com; bmasmith@ncsu.edu; dxlascel@ncsu.edu

a Comparative Pain Research Laboratory Department of Clinical Sciences, NCSU CVM, Raleigh, NC
b Farnam Companies, Inc., Phoenix, AZ
(Dr. Bledsoe’s current affiliation is with Nutramax Laboratories, Inc., Lancaster, SC)
c Department of Statistics, NCSU, Raleigh, NC
d Center for Comparative Medicine and Translational Research, NCSU, Raleigh, NC
e Center for Pain Research and Innovation, UNC School of Dentistry, Chapel Hill, NC

Extended duration analgesics for cats undergoing surgery would facilitate managing perioperative pain. We hypothesized that an extended release formulation of buprenorphine HCL (ER-Bup) would improve kinetic measures collected from cats in a unilateral onychectomy model compared to placebo in a novel experimental paradigm.

Using a blinded, randomized, two period crossover design, four cats were allocated to placebo (saline) or ER-Bup (0.6 mg/kg, subcutaneously) treatment groups, in random order. All animals underwent a unilateral forelimb onychectomy per period with a recovery/washout period between. Observational pain scores and kinetic data (using a pressure sensitive walkway) were collected prior to (baseline) and at intervals for 72 hours following surgery. Symmetry indices were derived for kinetic variables (peak vertical force [PVF]; vertical impulse [VI]) of each forelimb for landing following a jump from an elevated perch (0.7m) and for walking. Data were evaluated using a mixed model statistical approach.

No cats required rescue analgesics based on subjective pain score. PVF and VI of the operated limb were significantly decreased for both landing (p<0.0001 and p<0.0001) and walking (p<0.0001 and p<0.0001) following placebo. ER-Bup resulted in significantly decreased asymmetry in limb use during landing (PVF, p<0.0001; VI, p<0.0001) and walking (PVF, p=0.0002, VI p<0.0001). Landing data was easier to collect and appeared to better distinguish between groups.

This study demonstrates that SC ER-Bup may be an effective analgesic for a 72-hour period postoperatively. Furthermore, landing onto a PSW may be a useful and efficient way to assess analgesics in cats with forelimb pain.

This study was funded by Farnam Companies Inc. Phoenix, Arizona.
DO MAREK’S DISEASE VACCINES PROTECT AGAINST MAREK’S DISEASE VIRUS-INDUCED IMMUNOSUPPRESSION?

Author: Nik Faiz - graduate student

Coauthors: Aneg Cortes, Melissa West, James Guy, Tom Cimino, Isabel Gimeno

nmnikmoh@ncsu.edu
NCSU CVM

Marek’s disease virus (MDV) can induce severe immunosuppression in chickens. The negative consequences of MDV induced immunosuppression (MDV-IS) are difficult to assess at field level. Recently, we have developed a model to reproduce MDV-IS under laboratory conditions. In this model, the ability of MDV to reduce the efficacy of other vaccines, in particular of infectious laryngotracheitis (LT) vaccines, is measured. Our previous results showed that infection with very virulent plus (vv+) MDV at day of age resulted in total abrogation of the immune responses elicited by LT vaccines in commercial broilers bearing maternal antibodies against MDV. MDV-IS occurred in absence of gross tumors or lymphoid organs atrophy. In addition, vaccination at day of age with current available vaccines (HVT, HVT+SB1, and CV1988) did not protect against MDV-IS. The objective of this study was to evaluate the ability of various MD vaccination protocols including in ovo vaccination (HVT + CVI988) and double vaccination (HVT +SB1 in ovo followed by CVI988 at day of age) to protect against MDV-IS. We have also evaluated an experimental Marek’s disease vaccine (rMd5ΔMeq) for the ability to protect against MDV-IS. rMd5ΔMeq has been reported to confer the highest protection against vv+MDV and it did not produce immunosuppression by itself in previous studies using this model. Our result shows that current vaccines does not protect against MDV-IS and rMd5ΔMeq is a potential candidate for future MD vaccine. Result of this study will enhance our knowledge on how current control of Marek’s disease is protecting against MDV-IS.

Funding source: NCSU Faculty Research & Professional Development Fund
Development of antimicrobial resistant (AMR) bacteria within cattle is a food safety concern, yet it is unclear how treatment regimens influence AMR in enteric bacteria. The impact of treatment regimen on selection for AMR bacteria was examined through a pharmacokinetic analysis of two approved dosing schedules for enrofloxacin (Baytril): high dose (12.5 mg/kg) single injection and low dose (5 mg/kg) repeated injection every 24 hours for 3 days. Our hypothesis is that a single dose of enrofloxacin will achieve a greater maximum concentration of active drug within the gastrointestinal (GI) tract resulting in a lower concentration of AMR *E. coli*. Enrofloxacin and its metabolite ciprofloxacin were measured in GI lumen by collecting unbound drug using ultrafiltration probes surgically implanted into the ileum and spiral colon of 6 steers. *E. coli* was enumerated in ileal (collected using a unique modified ultrafiltration probe within the ileum) and fecal samples during and after treatment. Minimum inhibitory concentrations were determined using microbroth dilution assays for 8 *E. coli* isolates from each fecal sample. Fecal cultures indicated enrofloxacin treatment caused a significant reduction of *E. coli* concentrations in the feces during both treatment regimens. The counts returned to baseline after 3-4 days post treatment. The MIC values increased in one group of steers with both treatments, while the MIC value did not change in the other group with either treatment. Determination of the antibiotic concentration in the GI tract of these steers may explain these differences in change in MIC.

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EFFECT OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AND STEM CELL SUPERNATANT ON EQUINE CORNEAL WOUND HEALING IN VITRO

Jennifer Fowler, Veterinary Student
Amanda Sherman, Lauren Schnabel, Brian Gilger
Jmfowle3@ncsu.edu, absherma@ncsu.edu, lvschnabel@ncsu.edu, bgiler@ncsu.edu

Affiliation: North Carolina State University, College of Veterinary Medicine

Abstract:

Introduction: Corneal ulceration is a common problem in horses due to the prominence and location of the globe, and complications such as corneal infection can ultimately lead to enucleation and loss of performance. A therapeutic intervention that improves corneal wound healing has the potential to decrease this risk of infection. Application of mesenchymal stem cells (MSCs) has been shown to improve corneal healing capacity in rats and rabbits by increasing survival and proliferation of limbal epithelial cells. The purpose of this study is to compare the effects of autologous MSCs and MSC supernatant (MSC-Sp) on proliferation and wound healing capacity of equine corneal epithelial cells and keratocytes. We hypothesize that both MSCs and MSC-Sp will increase cellular proliferation and decrease epithelial wound healing time.

Methods: Six horses will undergo sternal bone marrow aspiration with isolation and culture of MSCs. Corneal epithelial cells and keratocytes will be also be collected and isolated to create corneal cell cultures. The in-vitro corneal scratch assay will be used to quantify cellular ingrowth. Cells will be treated with an MSC suspension, MSC-Sp solution, control (naïve culture media) or 0.1% dexamethasone solution (negative control) for 24 or 48 hours.

Results: We expect that cells treated with MSCs and MSC-Sp will have improved proliferation and a decrease in wound healing time compared to control naïve culture media and dexamethasone-treated cells.

Conclusions: If favorable results are obtained, further in-vivo studies are warranted to evaluate clinical potential. A non-cellular preparation such as MSC-Sp would be especially useful as a non-immunogenic “off-the-shelf” product.

Funding Sources: ACVO Vision For Animals Foundation
Macrophages have been identified within the corpus luteum (CL) of multiple species. They are present throughout the estrous cycle, and appear to play critical roles in CL development, maintenance and regression. Since luteal macrophages exist in a progesterone-rich environment (progesterone is a major product of CLs), we hypothesized that progesterone regulates macrophage function. In Experiment 1, the effects of progesterone on porcine CL Derived Macrophages (CLDM) were examined in vitro. In Experiment 2, CLDM were examined for the expression of genomic (PGR), and membrane-associated (mPRs), progesterone receptor, mRNAs. Mid-cycle porcine CL were dissociated with collagenase and macrophages, isolated. In Experiment 1, CLDM (~0.5 x 10^6/ml) were cultured for 24h with progesterone (0, 0.05, 0.5, 5 µg/ml), collected for RNA extraction, and analyzed for cell surface marker (CD 40, CD 68, CD 163) and cytokine (TNF-α, IL-1B, IL-6, IFN-γ, TGF-β, IL-10) mRNAs by Q-PCR. In Experiment 2, CLDM were subjected to RNA extraction and quantification of PGR and mPRs (PAQR5, PAQR7, PAQR8, PGRMC1 & PGRMC2) mRNAs by Q-PCR. In Experiment 1, progesterone decreased cell surface marker (2-5 fold) and cytokine (2-3 fold) mRNA levels in CLDM. In Experiment 2, CLDM expressed PGR and mPR mRNAs; PGRMC1 (100 arbitrary relative units) > PGRMC2 (28) > PGR (1.6) > PAQR5 (0.8) > PAQR8 (0.7) > PAQR7 (0.4). In summary, progesterone inhibited cell surface marker and cytokine mRNA by porcine CLDM, and CLDM expressed PGR and mPR. These data suggest that progesterone may play a key role in regulating luteal macrophages in vivo.

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ULTRASOUND TO QUANTITATIVELY ASSESS LEAN MUSCLE MASS IN COMPANION PETS

Amanda Fox, veterinary student, arfox2@ncsu.edu
Candace Matthews; Lindsey Bullen, DVM; Korinn Saker, MS, DVM, Ph.D., DACVN
cnmatth2@ncsu.edu; lereitz@ncsu.edu; kesaker@ncsu.edu

Department of Molecular Biomedical Sciences, North Carolina State University College of Veterinary Medicine

The current method of evaluating muscle condition in companion animals is a subjective score system (0-3), of limited clinical value. It is important to recognize lean muscle mass (LMM) loss early, as it hinders strength, mobility, immunity, and wound healing. Computed tomography (CT) is used in humans to quantitatively assess LMM, but is cost-prohibitive and not clinically applicable for companion pets. Therefore, our project aims to assess the value of ultrasound (US) as a quantitative technique to measure LMM of pets in a clinical setting.

We hypothesized there would be no difference between LMM measures obtained by a trained technician via ultrasound, compared with direct muscle dissection (MD), and that radiologist-obtained ultrasound measurements would correlate with CT-scan measures. Measures for these comparisons were obtained from cadavers and hospitalized patients scheduled for CT for various health issues. Canine cadavers were utilized for MD and US, and identified the temporalis, supra- and infraspinatus, and epaxial (between L2-L4) muscles as clinically useful locations. US measurements differed from the dissected LMM thickness by an average of 0.22 cm (± 0.24 cm). Ultrasound and CT measures of these same muscle locations, were subsequently obtained by a radiologist on hospitalized dogs, with owner consent. The US and CT measures differed by 0.07 cm (± 0.16 cm). Data from our preliminary study indicate that MD, US and CT measures of LMM thickness in dogs are similar; and suggest that US has promise as a valid tool to quantify LMM changes in companion pets.

This project supported by a grant from the Morris Animal Foundation Veterinary Student Scholars Program
EFFECTS OF PROSTAGLANDIN INHIBITION ON MUCOSAL RECOVERY FOLLOWING ISCHEMIC INJURY OF THE PORCINE JEJUNUM

Hannah Gardner, Veterinary Student (Class of 2018)
Vassili Kouprianov, Amanda Ziegler, Tiffany Pridgen, Anthony Blikslager
Contact: hegardne@ncsu.edu, atbliksl@ncsu.edu
Affiliation: Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University
Funding Source: CALS-CVM Dean’s Enrichment Grant, Equine Health Program

Diseases that affect the intestinal epithelium, such as ischemia/reperfusion and infectious diseases such as PEDv, are destructive to the intestinal mucosa. Epithelial restitution of denuded villi is a critical factor in re-gaining intestinal barrier function. After ischemic intestinal injury, prostaglandins facilitate the recovery process. Our aim was to study the role of prostaglandins on villus recovery by treating the tissue with the non-steroidal anti-inflammatory drug (NSAID) indomethacin, a prostaglandin-inhibitor. We hypothesized that time dependent mucosal injury will recover by a prostaglandin-mediated mechanism. Four Yorkshire cross piglets, 6-8 weeks old, were anesthetized. Sections of jejunum were subjected to 0, 30 or 45 minutes of ischemia and were removed at euthanasia. Mucosa was dissected from serosa and placed in Ussing chamber for a 180-minute recovery period. Trans-epithelial electrical resistance and mucosal-to-serosal flux of 3H-mannitol were measured as parameters of mucosal barrier function. Results showed a significant increase in the TER of the 45 and 30-minute untreated tissue at time point 120 through 180 minutes compared to the initial resistance. The TER of 45-minute indomethacin-treated tissue was significantly lower than that of the untreated 45-minute samples at these time points. The flux results showed that 45-minute untreated-ischemic tissue had a greater concentration of mannitol crossing from the mucosal to the serosal surface compared to the control sample. Inhibition of prostaglandins with an NSAID inhibits intestinal recovery following ischemic injury. Future studies will determine if there is an age-dependent effect on prostaglandin levels and associated epithelial repair.
PARTITIONING OF DRUGS AND CHEMICALS DURING TWO CHEESE MAKING PROCESSES

Leland S. Garrett, veterinary student
James D. Brooks, Ronald E. Baynes
lsgarret@ncsu.edu, jdb@ncsu.edu, Ronald_Baynes@ncsu.edu
Center for Chemical Toxicology Research and Pharmacokinetics, NCSU CVM

The FDA has set tolerance limits for drugs in bulk tank whole milk. However, little is known about partitioning of drugs into various milk fractions. To make cheese, casein curd must be separated from liquid whey, and two different methods to accomplish this are often utilized by artisanal cheese makers. One method requires acid and the other requires rennet, a complex of enzymes produced in the stomach of ruminant animals. Most cheeses in the United States are produced with the latter method. During cheese making, drugs might partition into the milk fat, whey, or curd. Predicting the degree of binding to either casein or whey proteins is an important issue to food safety and human health. Current regulations do not address the binding properties of drugs found in milk during the cheese making process. This study aims to estimate the binding of drugs to casein proteins using liquid scintillation counter (LSC) analysis. Pilot studies employed radiolabeled agrichemicals (4-Cyanophenol and Diazinon) selected according to their location in a size/solubility chemical space. Molecular weight (119.12 and 304.35, respectively) and octanol:water partition coefficient (logKow, 1.6 and 3.81) were used as selection criteria. Whole milk and skim milk cheeses were tested. Curd:whey partition coefficient (logKcw) was determined to compare with LogKow values. LogKcw values differed per curd separation method: Acid-based separation: -0.38555 and -0.22381; Rennet-based separation: 0.5092 and 1.8038, respectively for 4-Cyanophenol and Diazinon. Further pilot drug testing will verify whether LogKow could be a predictor for partitioning behaviors.
THE ROLE OF TRIM9 IN MACROPHAGE MIGRATION IN DEVELOPING
ZEBRAFISH (DANIO RERIO) IN RESPONSE TO A BACTERIAL AGONIST

Jamie Gerlach- Veterinary Student
Debra Tokarz, Jeffrey Yoder
jgerlac@ncsu.edu
Affiliation: NCSU CVM

Abstract:

Leukocyte migration is a critical component in the immune response to infection, but the intracellular mediators of migration are not well understood. Trim9 is a gene that responds to immune stimulation in both zebrafish and human neutrophils and macrophages. Although it has no previously reported immune function, its published role in axon migration suggests it may also function in leukocyte migration. Our lab has successfully demonstrated that expressing a truncated version of trim9, which lacks a functionally important RING-domain, negatively affects the ability for zebrafish macrophages to migrate to sites of inflammation in response to a viral mimic. Overexpressing full-length trim9 in zebrafish macrophages has no evident effects on macophages migration during exposure to the viral mimic. This suggests that trim9 plays a previously unknown role in macrophage chemotaxis. Based on the response to the viral mimic, we hypothesized that macrophages expressing RING-deleted trim9 would also have migration deficits to a bacterial agonist. To test this, Pam3CSK4, which mimics bacterial lipopeptides, was injected into the otic vesicles of 4-day-old zebrafish expressing either trim9 transgene or a control transgene, within macrophages. With the use of fluorescent imaging, migration assays were performed to determine a migration index for macrophages expressing each transgene. The results show that migration of macrophages in response to a bacterial mimic is not affected in those zebrafish with overexpressed full-length trim9, but is negatively affected in zebrafish expressing RING-deleted trim9. These results further support a role for Trim9 in mediating macrophage chemotaxis.

Funding source: T-35 IBRTP, Grant # NIH T35OD011070 & T32 OD011130
THE EFFECT OF REMOVING CHLORHEXIDINE SCRUB FROM VETERINARY SURGEON USE ON THEIR CARRIAGE OF ANTISEPTIC AND ANTIMICROBIAL-RESISTANT STAPHYLOCOCCUS SPECIES

Ericka Gonzalez  
Veterinary Student  
Anna Rogers, Kyle Mathews, and Megan Jacob  
Egonza3@ncsu.edu; anna_rogers@ncsu.edu; kmathews@ncsu.edu; mejacob@ncsu.edu  
North Carolina State University, College of Veterinary Medicine, Raleigh, NC

Chlorhexidine has long been used as an antiseptic in veterinary medicine and continues to be used as a pre-surgical hand scrub. However, long-term use of chlorhexidine may confer resistance to bacterial populations. We aimed to determine the prevalence of antiseptic and antibiotic resistance in *Staphylococcus* associated with the nares and hands of veterinary surgeons before after transitioning from a chlorhexidine scrub to an alcohol-based rub prior to performing surgery. Clinicians at the NCSU Veterinary Hospital were recruited to participate in the study. Nares and hand swabs of participants were collected at three-month intervals. Participants were sampled at time 0 (n=26), and at 3 (n=16) and 6 (n=17) months post switch. The swabs were cultured for *Staphylococcus* species and isolates were identified using MALDI-TOF mass spectrometry. Polymerase chain reaction was used to evaluate the presence of mecA, qacA/B, and smr, genes associated with antimicrobial and antiseptic resistance. Antimicrobial and chlorhexidine minimum inhibitory concentrations of isolates were determined by broth microdilution. Results showed a decrease in the proportion of hand *Staphylococcus* isolates positive for mecA and qacA/B genes from 0 months (84.6% mecA, 93.3% qacA/B) to 3 (38.5% mecA, 44.4% qacA/B) and 6 (55.6% mecA, 55.5% qacA/B) months. Similarly, the proportion of nares *Staphylococcus* isolates positive also decreased from 0 months (78.2% mecA, 53.8% qacA/B) to 3 (75% mecA, 31.2% qacA/B) and 6 (62.5% mecA, 50% qacA/B) months. Further characterization of isolates is on-going. Our early results indicate that removing the use of chlorhexidine may also correspond to less antiseptic and antibiotic resistance in *Staphylococcus* isolates of veterinary surgeons.
QUANTIFICATION OF THERMAL AND MECHANICAL SENSORY THRESHOLDS IN CHONDRODYSTROPHOID DOGS

Angela Gorney: veterinary student
Carly Dohse, Sarah Blau, Kim Williams, Ji-Hey Lim, David Knazovicky, B. Duncan X. Lascelles, and Natasha J. Olby
agorney@ncsu.edu, njolby@ncsu.edu

NC State University CVM, Raleigh, NC

Chondrodystrophic dog breeds such as the Dachshund have a high incidence of acute intervertebral disc herniations causing thoracolumbar spinal cord injury (SCI), paralysis and sensory loss. We hypothesized that quantification of hind limb sensory thresholds following SCI would document neurologic recovery. The aims of this study were to establish the thermal and mechanical sensory thresholds in normal and SCI dogs and to determine if there was a difference in sensory thresholds between these two groups. Thermal testing was performed by placing a hot (49°C) and cold (5°C) probe on the skin of the dorsal metatarsal region, and mechanical thresholds were tested using calibrated forceps to apply pressure to the lateral digit of each hind paw. Trials were conducted until evidence of conscious perception, including escape behavior, vocalization, and orientation towards the stimulus, or the maximum limit was reached. Thresholds were compared using a Wilcoxon rank sum test. Thermal testing was performed in 15 normal and 16 SCI dogs; thresholds were significantly different between the two groups for both hot and cold (P<0.0001). Mechanical thresholds were tested in 13 normal and 17 SCI dogs and were significantly different (P=0.0024). SCI dogs were grouped as paraplegic without nociception, paraplegic, non-ambulatory paraparetic, and ambulatory paraparetic. Thermal and mechanical thresholds were significantly different among these groups (P<0.05). We conclude that sensory thresholds in dogs are altered by SCI. The differences in sensation among neurologic grades indicate that these techniques can be used to further characterize recovery of SCI dogs.

Funding Sources: Center for Comparative Medicine and Translational Research, Department of Defense, Merial, Morris Animal Foundation
Chiari-like malformation is present in a majority of Cavalier King Charles Spaniels (CKCS). This developmental disorder results in an abnormally small caudal fossa, causing obstruction of the foramen magnum by the cerebellum, altering cerebrospinal fluid flow and producing a syringomyelia (SM) in the spinal cord. The SM centers on the dorsal grey matter, producing neuropathic pain. We hypothesized that mechanical sensory thresholds would be decreased in CKCS with SM. The study aims were to: 1) determine whether sensory thresholds could be identified by an increase in heart rate, and 2) quantify mechanical thresholds in CKCS and compare thresholds in dogs with and without SM. Mechanical sensory thresholds were tested using calibrated forceps to apply pressure until conscious perception or a maximum pressure of 4.0 kg was reached. An elastic band with a Polar Heart Rate Sensor was placed around the thorax of the dog during testing and the heart rate collected telemetrically. Nineteen dogs were evaluated, 12 of which had SM confirmed by magnetic resonance imaging. The heart rate monitor generated reliable data, but the heart rate proved extremely labile and could not be used to identify sensory thresholds. Thresholds were therefore determined by behavioral responses alone. In dogs without SM, the mean mechanical thresholds were 0.45kg (digit) and 2.64kg (neck) compared with 0.47kg (digit) and 2.40kg (neck) in dogs with SM, and were not statistically different between groups. Testing of additional dogs is ongoing to increase sample size and allow correlation of thresholds with maximum SM height.

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COMPARISON OF SERUM AND PLASMA VALUES IN MULTIPLEXED CYTOKINE MEASUREMENT IN CATS

Margaret Gruen\textsuperscript{a,b} – Graduate student
Kristen Messenger\textsuperscript{c}, Emily Griffith\textsuperscript{d}, Hayley Paradise\textsuperscript{a}, Shelly Vaden\textsuperscript{e}, B. Duncan X. Lascelles\textsuperscript{a,b,f}
\texttt{megruen@ncsu.edu, kmmessen@ncsu.edu, eghohmei@ncsu.edu, hparadi@ncsu.edu, shelly_vaden@ncsu.edu, duncan_lascelles@ncsu.edu}

Affiliations:
a \textit{Comparative Pain Research Laboratory, Department of Clinical Sciences, NCSU CVM, Raleigh, NC}
b \textit{Center for Comparative Medicine and Translational Research, NCSU, Raleigh, NC}
c \textit{Molecular Biomedical Sciences, NCSU CVM, Raleigh, NC}
d \textit{Department of Statistics, NCSU, Raleigh, NC}
e \textit{Department of Clinical Sciences, NCSU CVM, Raleigh, NC}
f \textit{Center for Pain Research and Innovation, UNC School of Dentistry, Chapel Hill, NC}

Abstract:

Degenerative joint disease (DJD) is highly prevalent in cats, and pain is a common feature contributing to morbidity. In humans, alterations of cytokine levels have been associated with pathological joint deterioration and pain. Similar changes have not been investigated in cats, and would be of great interest as biomarkers of DJD and associated pain. Cytokine levels can be conveniently measured using multiplex technology and small samples of serum or plasma. However, serum and plasma are not interchangeable for most bioassays, with general recommendations to consistently use one. Correlations for cytokine levels between matched serum and plasma have not been evaluated in cats. The objective of this pilot study was to evaluate the levels of detection and agreement between paired serum and plasma samples in cats.

Paired serum and plasma samples were obtained from 38 cats. Blood was collected into anti-coagulant free and EDTA Vacutainer\textsuperscript{®} tubes, serum or plasma extracted, and samples frozen at -80°C until testing. Duplicate samples were tested using a 19-plex feline cytokine/chemokine magnetic bead panel. Coefficients of variation were low, with 2.6\% of results having CVs greater than 20\%. Results from >50\% of cats were below the limit of quantification for both serum and plasma for 7 analytes, and for plasma only for an additional 3 analytes. Agreement between serum and plasma was high, with correlation coefficients all exceeding 0.91. Mean serum values were higher than plasma values for 84\% of analytes. While serum and plasma agreement was generally good, detection was improved using serum samples.

\textit{Funding Source: Winn Feline Foundation provided the funds for the study. Margaret Gruen receives funding support from the National Institutes of Health Ruth L. Kirschstein National Research Service Award (T32OD011130).}
VETERINARIAN ATTITUDES TOWARD CLINICAL RESEARCH: A SURVEY STUDY

Margaret Gruen\textsuperscript{a,b} – Graduate student  
Mark Rishniw\textsuperscript{c,d}, B. Duncan X. Lascelles\textsuperscript{a,b,e}  
megruen@ncsu.edu, mr89@cornell.edu, Duncan_lascelles@ncsu.edu  

Affiliations:  
\textsuperscript{a} Comparative Pain Research Laboratory, Department of Clinical Sciences, NCSU CVM, Raleigh, NC  
\textsuperscript{b} Center for Comparative Medicine and Translational Research, NCSU, Raleigh, NC  
\textsuperscript{c} Clinical Sciences Department, Cornell University CVM, Ithaca, NY  
\textsuperscript{d} Veterinary Information Network, Davis, CA  
\textsuperscript{e} Center for Pain Research and Innovation, UNC School of Dentistry, Chapel Hill, NC  

Abstract:  
Enrollment in clinical research trials is hindered by low recruitment rates, extending timelines and costs for research and limiting generalizability of findings. In veterinary medicine, recruitment includes pet owners as well as pets. Previous research showed recommendation from a veterinarian matters to cat owners considering participation in clinical trials. However, veterinarians’ attitudes toward clinical research, and the factors influencing participation in and recommendation of clinical trials are unknown. The objective of this internet-based survey study was to identify factors influencing veterinarians’ willingness to recommend clinical trials, including barriers to participation.  

Six hundred and fifty-two respondents had graduation years representative of currently practicing veterinarians. The majority were general practitioners seeing small animals, +/- exotic species. Participation in clinical research was low, with 82% of respondents spending no time in clinical research. Respondents were more likely to recommend trials for novel therapies and those sponsored by academic institutions. Barriers to participation included distance to clinical trial centers, time restrictions, and lack of awareness of available clinical trials, with 30% responding that they do not usually hear about clinical trials. Veterinarians likely underestimate the impact of their recommendation on their clients’ participation, though the majority of respondents rated their recommendation as important.  

Overall participation was low, making generalizations difficult. Conclusions based on the responses received show participation in clinical research is low among veterinarians, and awareness of available clinical trials could be increased. The survey identified factors that are important in clinical trial design and could increase participation from veterinarians.  

Funding Source: Margaret Gruen receives funding support from the National Institutes of Health Ruth L. Kirschstein National Research Service Award (T32OD011130).
AN ANTI-NERVE GROWTH FACTOR MONOCLONAL ANTIBODY (NV-02) IMPROVES ACTIVITY AND DECREASES OWNER RATINGS OF MOBILITY IMPAIRMENT IN CATS WITH DEGENERATIVE JOINT DISEASE ASSOCIATED PAIN

Margaret Gruen\textsuperscript{a,b} – Graduate student
Andrea Thomson\textsuperscript{a}, Hayley Paradise\textsuperscript{a}, David Gearing\textsuperscript{c}, B. Duncan X. Lascelles\textsuperscript{a,b,d}
megruen@ncsu.edu, aethomson@ncsu.edu, hparadi@ncsu.edu, dave.gearing@nexvet.com, duncan_lascelles@ncsu.edu

Affiliations:
\textsuperscript{a} Comparative Pain Research Laboratory, Department of Clinical Sciences, NCSU CVM, Raleigh, NC
\textsuperscript{b} Center for Comparative Medicine and Translational Research, NCSU, Raleigh, NC
\textsuperscript{c} Nexvet Biopharma, Melbourne, Australia
\textsuperscript{d} Center for Pain Research and Innovation, UNC School of Dentistry, Chapel Hill, NC

Anti-nerve growth factor (NGF) monoclonal antibodies have demonstrated potential for treatment of osteoarthritis-associated pain in humans and dogs. While critical for nerve growth during development, in adulthood, NGF serves a primarily nociceptive role, sensitizing peripheral neurons to painful stimuli. In this double-blind, randomized, placebo-controlled pilot proof-of-principle study, the safety and efficacy of a fully-felinized anti-NGF antibody (NV-02) were evaluated in 34 cats with naturally-occurring degenerative joint disease (DJD). We hypothesized that NV-02, administered subcutaneously, would improve owner ratings of mobility/activity impairment and increase activity in cats with DJD.

Enrolled cats were required to have joint pain on orthopedic exam, overlapping radiographic evidence of DJD, and owner-rated mobility impairment. After a 2-week baseline period, cats were administered either NV-02\textsubscript{high}, NV-02\textsubscript{low}, or placebo subcutaneously. Outcome measures included owner-completed clinical metrology instruments (CMIs) and objectively measured activity. Owners completed CMIs at baseline and every 3 weeks for 9 weeks after treatment; activity counts were collected each minute over the entire 11-week study period.

While a caregiver placebo effect was detected, greater improvement in CMI scores was seen in the treatment groups as compared to the placebo group 3 weeks after treatment. Cats in the treatment groups showed greater change (increase) in activity than cats in the placebo group for the 2-6 weeks following treatment. NV-02 was generally well-tolerated at both dosages.

NV-02 is a promising novel treatment for DJD-associated pain in cats. Future work will expand these findings and investigate efficacy in a larger group of cats given multiple treatments.

\textit{Funding Source: Funding for the study provided by NexVet Australia. Margaret Gruen receives funding support from the National Institutes of Health Ruth L. Kirschstein National Research Service Award (T32OD011130).}
CHARACTERIZATION OF LESION SEVERITY AFTER ISCHEMIA-REPERFUSION INJURY IN EQUINE STRANGULATED SMALL INTESTINE.

Esther Gu (veterinary student)
Leslie Smith, Liara Gonzalez, Anthony Blikslager
equ@ncsu.edu, Anthony_Blikslager@ncsu.edu

Department of Clinical Sciences (Gonzalez, Blikslager), College of Veterinary Medicine, North Carolina State University, Raleigh, NC

The presence and development of undetected mucosal ischemia-reperfusion (IR) injury at the margins of strangulating intestinal lesions may predispose horses to post-operative ileus and adhesions. However, the presence of reperfusion injury following clinical ischemia remains controversial. The aim of this study was to assess the degree of IR injury at resection margins of clinically strangulated small intestine using histomorphometry and immunohistochemistry. Samples of jejunum from proximal and distal margins of resected small intestine from 11 horses diagnosed with strangulated intestinal obstruction between 2013 and 2015 were evaluated using histomorphometry. Neutrophil infiltration and oxidative damage were assessed using immunohistochemistry. Villi were significantly longer in proximal margins than in hemorrhagic and ischemic sections of strangulated intestine (p=0.003). Hemorrhagic sections had significantly greater epithelial loss compared to distal margins, and both hemorrhagic and ischemic sections had significantly more epithelial loss than proximal margins (p<0.0001). We found no differences in crypt length amongst the different sections (p=0.8). Preliminary histological data show evidence of increased neutrophil activity and lipid peroxidation at the epithelial villus tips. Unexpectedly, the hemorrhagic sections of strangulating lesions have the shortest villi, greatest epithelial loss, and greatest degree of hemorrhage. The presence of increased oxidative damage at villus tips suggests evidence of IR injury. An understanding of the inflammatory processes involved in IR injury can lead to better strategies to reduce post-operative complications.

Student support: Interdisciplinary Biomedical Research Program (NIH T35OD011070)
THE USE OF PROSTAGLANDIN E-2 (PGE-2) RECEPTOR ANTAGONISTS FOR INDUCTION OF LUTEOLYTIC SENSITIVITY (LS) IN THE PORCINE CORPUS LUTEUM (CL): IN VITRO STUDIES.

E.K. Hall: Veterinary Student, A.M. Sosnowski, S. Frandsen, J.E. Gadsby

E-mail addresses: ekhall@ncsu.edu, jgadsby@ncsu.edu
Department of Molecular Biomedical Sciences, NCSU CVM

The porcine CL lacks luteolytic sensitivity (LS) to PGF-2α for the first 12-13 days of the estrous cycle, thus PGF-2α agonists are impractical for estrous cycle regulation or synchronization in this species. Since the porcine CL also synthesizes, and is responsive to, the luteotropic PG, PGE-2, during the first 12-13 days of the cycle, we hypothesize that this may account for the lack of LS during this period. The objective of this study was to examine the effects of different PGE-2 receptor (EP) antagonists (AH 6809, blocks EP-1, -2 and -3; SC-19220, blocks EP-1; L-798106, blocks EP-3; Duke Compound #8, blocks EP-3; and Duke Compound #10, blocks EP-3) on the sensitivity of porcine luteal cells to PGF-2α, in vitro. Porcine CL were collected from a local slaughterhouse at early (~days 4-7) or mid (~days 8-12) stages of the estrous cycle, luteal cells dissociated with collagenase and cultured for different times with various doses of EP antagonists (listed above) and PGF-2α. Following culture, spent culture media were assayed for progesterone by enzyme-immunosassay. Analyses of samples from these experiments are in progress and will be presented. From these studies, it is hoped that we will be able to identify an effective EP-receptor antagonist, which increases the sensitivity of luteal cells to PGF-2α, in vitro. Following these studies, we plan to progress to in vivo studies and demonstrate the use of the selected EP antagonist as a novel drug for the induction of LS and estrous cycle regulation in the pig.

This project was supported by, AFRI Grant no. 2012-67015-19349 from the USDA/NIFA (JEG), the Veterinary Scholars Program at CVM, NCSU (EH), and funds from the State of North Carolina (JEG).
EVALUATING THE IMPACT OF EARLY-TARGETED NUTRITION ON CLINICAL OUTCOMES FOR DOGS WITH SEVERE ACUTE PANCREATITIS

Jessica P. Harris¹ – House Officer
Nolie K. Parnell², Korinn E. Saker¹
Jess_Harris@ncsu.edu, parnelln@purdue.edu, Korinn_Saker@ncsu.edu
¹NCSU CVM, Raleigh, NC, United States of America
²Purdue University – CVM, West Lafayette, IN, United States of America

Human medicine guidelines advocate early enteral feeding for acute pancreatitis patients due to an association with better clinical outcomes as compared to patients held NPO. In veterinary medicine, no studies evaluate the impact of nutritional therapy timing on clinical outcome. This retrospective study aimed to determine whether provision of targeted (<3.0 grams/100 kcal) nutritional therapy to acute pancreatitis patients within 48 hours of admission (Early Feeding Group, EFG) resulted in decreased time to volitional intake, incidence of gastrointestinal intolerance (GI, regurgitation or vomiting), and total hospitalization time compared to patients held NPO (Delayed Feeding Group, DFG).

EFG dogs returned to volitional intake (RVI) faster compared to the DFG (p=0.05). DFG dogs experienced significantly increased (p=0.04) GI incidences while hospitalized with 71% of events occurring under an imposed NPO order or refusing to eat. The remainder of events were noted when consuming <66% RER. The average dietary fat level (grams/100 kcal) consumed by DFG dogs exhibiting GI was 3.6 (mode 5.4), compared to 3.3 (mode 2.9) for those with no GI. EFG dogs experiencing GI also had majority of events occur while refusing to eat, and the remainder of incidences noted when dogs consumed <66% RER. The average fat level consumed by EFG dogs with GI was 4.2 (mode 5.4) compared to 3.2 (mode 1.8) in those with no GI. Timing of feeding did not impact hospitalization time (p=0.8).

Study findings suggest early targeted nutritional therapy implementation (offering low-fat diet options within 48 hours of admission) had a positive impact on RVI and GI incidence. This suggests withholding targeted nutrition support may not benefit the acute pancreatitis patient.

Funding sources: None
QUESTING FOR EQUINE VECTOR-BORNE DISEASES BY SEROLOGICAL AND MOLECULAR METHODS

Author: Barbara C. Hegarty Research Associate,
Co-authors: Barbara Qurollo, Jeffrey Tyrrell, Kaitlin Haney, Edward B. Breitschwerdt

Email addresses: bhegarty@ncsu.edu, baquroll@ncsu.edu, jdtyrrell@gmail.com, knhaney@ncsu.edu, ed_breitschwerdt@ncsu.edu.
Affiliation: Vector Borne Disease Diagnostic Laboratory (VBDDL), NCSU-CVM,

Abstract:
Anaplasma phagocytophilum, Borrelia burgdorferi and Neorickettsia risticii (Potomac Horse Fever) are recognized vector-borne pathogens among horses. Additionally, Babesia, Bartonella, Ehrlichia, Rickettsia, Theileria and hemotropic Mycoplasma species have been detected in horses with clinical disease spectrums ranging from nonclinical to fatal. The goal of this pilot study is to investigate serological and molecular (PCR) tests that will detect vector-borne disease (VBD) pathogens in horses.

Hypothesis: A broad spectrum approach using diagnostic IFA/PCR panels applied to equine infectious diseases will develop a more definitive picture of vector-borne equine pathogens, including the influence of novel species and co-infections.

Study Populations:
Group I EDTA/serum sets from clinically healthy horses pastured in North Carolina to establish background exposure to, or infection with, VBDs.
Group II EDTA/serum sets from horses submitted for diagnostic testing to the VBDDL.

Methods: Serologic and PCR testing was performed retrospectively. The commercially available Snap® 4DX® Plus ELISA detects serum antibodies to 3 Ehrlichia species, 2 Anaplasma species and to Borrelia burgdorferi. ImmunoFluorescent Assays (IFA) were performed using available Babesia, Bartonella, Neorickettsia and Rickettsia antigens. PCR assays were used to detect DNA of 8 targeted genera.

Results: The combined use of serological and molecular assays in this study gave evidence of exposure to vector-borne pathogens, including Anaplasma, Bartonella, Borrelia, and Ehrlichia spp.

Conclusion: Horses are exposed to and can be infected with vector-borne pathogens that commonly infect dogs. This study supports the need to optimize VBD testing for horses.

Funding sources: ELISA kits were generously donated by IDEXX Laboratories, Inc. Westbrook, ME. Samples, supplies and reagents for IFA and PCR were made available through the revenues of the Vector Borne Disease Diagnostic Laboratory, NCSU.
SAFETY OF ALLOGENEIC CARDIOSPHERE-DERIVED CELLS IN CANINE DIALATED CARDIOMYOPATHY

Taylor Hensley: Research Associate
Co-authors: Bruce Keene, Kate Meurs, Jessica Ward, Kathleen Woodruff, Teresa DeFrancesco, Sandra Tou, Petra Vasilik, John Cullen, Tianxia Zhang, Ke Cheng PhD
mthensle@ncsu.edu, kcheng3@ncsu.edu
Affiliation: NCSU CVM

Background:
Cardiosphere-derived cells (CDCs) have been shown to reduce scarring after myocardial infarction, increase viable myocardium, and boost cardiac function in preclinical models. Allogeneic CDC cell infusion has also proven to be safe and viable in small animal models.

Methodology/Principal Findings:
Canine CDCs (cCDCs) were grown from a donor dog heart. The phenotype of the cCDCs were characterized by flow cytometry. Similar to human CDCs, cCDCs express CD105 and are slightly positive for ckit. In vitro, conditioned media from cCDCs reduced apoptosis of neonatal rat cardiomyocytes and boosted cell survival and contraction. 30 million of allogeneic cCDCs were infused into the coronary vessels of Doberman Pinscher dogs with dilated cardiomyopathy. Adverse events were closely monitored and cardiac functions were measured by echocardiography.

Conclusions:
No adverse events occurred during and after cell infusion. Given the small sample size, efficacy was not the primary endpoint of the study. Histology on dog hearts revealed that injected cells were not present in the heart after 1 month.
INDUCING METAMORPHOSIS IN THE BUDGETT’S FROG (LEPIDOBATRACHUS LAEVIS), A NEW AMPHIBIAN MODEL WITH CANNIBALISTIC TADPOLES

Margaret Hull, veterinary student
Nirav Amin, Gregory Lewbart and Nanette Nascone-Yoder
mahull@ncsu.edu, namin@ncsu.edu, galewbar@ncsu.edu, nmnascone@ncsu.edu
Affiliations: NCSU CVM

The Budgett’s frog (Lepidobatrachus laevis) is a South American aquatic frog with large, rapidly-developing embryos, making it potentially superior to the common laboratory amphibian, Xenopus laevis, as a model for studying embryonic development. Unlike most frogs, Lepidobatrachus generate cannibalistic tadpoles that, in the wild, subsist on a diet of their siblings. However, when raised in the laboratory on a diet of slower-growing Xenopus tadpoles, many Lepidobatrachus fail to undergo the last phase of metamorphic transformation, suggesting that a specific factor from ingested siblings is required to achieve froglet stage. Since metamorphosis is regulated by thyroid hormone, we hypothesized that Lepidobatrachus normally obtain supplemental thyroid hormone from their unusual diet.

To test this hypothesis, prometamorphic Lepidobatrachus tadpoles (forelimb emerging) were either directly immersed in 1ug/mL thyroxine for 72 hours, or were fed a diet of other “thyroxine-loaded” Xenopus or Lepidobatrachus tadpoles. Individual tadpoles in each group were assessed every 24 hours for metamorphic climax, as indicated by complete tail regression.

Preliminary results indicate that higher rates of complete metamorphosis were indeed induced by consuming thyroxine-loaded prey. However, thyroxine-loaded Lepidobatrachus tadpoles were more effective at inducing metamorphosis than thyroxine-loaded Xenopus tadpoles, suggesting that cannibals obtain additional, thyroid-independent advantages from eating their siblings. Although direct thyroxine immersion also accelerated metamorphic transformations, this treatment was associated with high morbidity and mortality and is therefore not a viable option. Our results suggest simple improvements for successfully rearing Lepidobatrachus in the laboratory, in order to facilitate wider utilization of Lepidobatrachus as a new amphibian model.

Funding: NCSU CVM Veterinary Scholars Program
IDENTIFICATION OF TWO NOVEL MYCOPLASMA SPECIES IN AN EASTERN BOX TURTLE (TERRAPENE CAROLINA CAROLINA) AND YELLOW-BELLIED SLIDER (TRACHEMYS SCRIPTA SCRIPTA)

JoAnna Jarred, Veterinary Student, JbJarred@ncsu.edu - North Carolina State University
Kelsey Stover – kestover@ncsu.edu, Gregory Lewbart, MS. VMD, DACZM – galewbar@ncsu.edu, Ricardo Maggi, MS. PhD – rgmaggi@ncsu.edu & Edward Breitschwerdt, DVM, DACVM - ed_breitschwerdt@ncsu.edu

Work was performed in the Vector Borne Disease Diagnostic Laboratory at North Carolina State University’s College of Veterinary Medicine.

The purpose of this study was to determine if turtles from the Turtle Rescue Team (TRT) at North Carolina State University are carriers of hemotrophic Mycoplasma and/or Bartonella spp. Species of Mycoplasma have previously been identified in several turtle and other reptilian species. TRT is a student ran organization that rehabilitates native aquatic and terrestrial species of North Carolina turtles. Spleen samples were collected on necropsy from 53 specimens representing seven species. Samples were collected from May 2014-July 2014 with subjects mainly originating from central North Carolina. The turtles were dead or euthanized upon arrival from traumatic injuries, or died shortly after beginning treatment in the TRT clinic. Spleen tissue was harvested on necropsy and DNA was extracted using a commercially available kit. A polymerase chain reaction (PCR) test was then performed for both species of bacteria. All samples tested negative for Bartonella spp. Two samples tested positive for different novel species of Mycoplasma via detection and sequencing of the 16S rRNA subunit. These novel species show striking similarities to pathogenic strains in other species. Pathogenic strains of bacteria are an important part of the ecosystem health of North Carolina, and diversity of reservoir hosts may be greater than previously thought.

Funding for this project was generously provided by The Robert J. Koller Aquatic Animal Medicine Research Endowment.
THE ROLE OF SULFATION ON ASYMMETRIC CELL DIVISION IN TYPE II ALVEOLAR EPITHELIUM: CREATING AN IN VITRO MODEL

JoAnna Jarred, Veterinary Student, JbJarred@ncsu.edu – North Carolina State University
Donna Newman PhD – drnewman@ncsu.edu, Philip Sannes PhD – plsannes@ncsu.edu, Kenneth Adler, PhD – Kenneth_adler@ncsu.edu

Work is performed in the laboratory of Dr. Philip Sannes at North Carolina State University’s College of Veterinary Medicine.

Alveolar type II epithelial (ATII) cells produce surfactant in the pulmonary alveolus and function as stem cells for type I (ATI) epithelial cells in order to promote homeostasis and aid in cellular repair processes after injury. To accomplish the latter, it’s been proposed that ATII cells must asymmetrically divide to efficiently differentiate by segregation of relevant signaling factors. It is hypothesized that the interactions of ATII cells with specific components of the extracellular matrix (ECM), in particular sulfated molecules, play a key role in determining the fate of ATII cells. To model the known in vivo differences in ECMs between ATII and ATI cells, we are interested in determining if cell signaling factors segregate in dividing cells at the interface between high vs. low sulfated substrata. An in vitro model of the ECM interface was created using Type I collagen, carbon black molecules, and sulfated/desulfated heparin. After appropriate manipulation clear demarcation of the interphase between matrix types can be seen. A549 cells (alveolar adenoma cells) were seeded on the matrices. Immunohistochemical staining was performed for NUMB, Notch, PCNA, and Ki67 on the A549 cells – with the most favorable responses being seen on NUMB, Notch, and Ki67. Now that an appropriate in vitro model has been created, further evaluation of the cellular response of isolated normal human ATII cells is underway. Immunofluorescence will be used to define segregation of selected cell signaling factors at the matrix interface to gain a better understanding of the role of sulfation in asymmetric cell division.

Funding was provided by the Interdisciplinary Biomedical Research Program through the National Institute of Health through grant T35OD011070.
CIC-2 REGULATES COLONIC EPITHELIAL HOMEOSTASIS, TUMORIGENICITY AND METASTATIC BEHAVIOR IN COLITIS-ASSOCIATED COLORECTAL CANCER

Younggeon Jin: Postdoctoral Research Scholar
Dina Ibrahim, Anthony T Blikslager
Email Address: yjin8@ncsu.edu

Center for Comparative Medicine and Translational Research, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

The CIC-2 chloride channel has a critical role in the regulation of tight junction barrier in several intestinal disease states, including experimental ischemia/ reperfusion injury and inflammatory bowel disease. Our recent studies have suggested that CIC-2 is also associated with intestinal epithelial cell proliferation and differentiation during colitis. Disruption of tight junction (TJ) proteins is a hallmark of epithelial-mesenchymal transition (EMT) signaling. Thus, we hypothesized that CIC-2 would have a role in colonic epithelial homeostasis, tumorigenecity and EMT-associated metastasis during development of colitis-associated colorectal cancer by altering tight junction proteins. The effect of the absence of CIC-2 on proliferation and differentiation was determined in CIC-2+/+ and CIC-2−/− mice. Colitis-associated colorectal cancer (CAC) was induced with azoxymethane (AOM) followed by cyclical administration of dextran sulfate sodium (DSS). CIC-2−/− mice had an increased colonic epithelial turnover rate and evidence of reduced colonocyte differentiation. CIC-2−/− mice subjected to AOM/DSS treatment to model CAC had an increased tumor number as well as increased incidence of high grade dysplasia as compared to CIC-2+/+ mice. Total expression of the TJ proteins occludin and claudin-1 was significantly reduced in the CIC-2−/− mice with colorectal tumors. Deletion of CIC-2 stimulated EMT signaling during CAC development, as indicated by upregulated β-cadherin and vimentin. CIC-2−/− mice colorectal tumors also showed significant reductions of the expression of the epithelial marker EpCAM and E-cadherin compared to the CIC-2+/+ mouse colorectal tumors. Collectively, these results indicate that CIC-2 is associated with regulation of TJ proteins and EMT signaling in murine colitis-associated tumor formation.
Neurotoxic effects occur in people following high dose manganese (Mn) exposure. Several physiologically-based pharmacokinetic (PBPK) models useful for Mn risk assessment have been developed; however, these models primarily focus on inhalation and dietary exposure. This study addresses PBPK model data gaps concerning the pharmacokinetics of ingested Mn following non-dietary exposure. Adult male rats were allocated to control diet (10 ppm Mn), high Mn diet (200 ppm Mn), Mn-contaminated drinking water, and Mn gavage treatment groups. Animals in the drinking water and gavage groups were given the 10 ppm Mn diet and supplemented with MnCl2 in drinking water or once-daily gavage to provide a daily Mn intake equivalent to that seen in the high Mn diet group. Mean dietary and body weight data were used to adjust weekly Mn intake rates for these groups. Mean body weight changes seen between groups suggest that oral Mn exposure slows body weight gain. Rats were anesthetized with ketamine and xylazine following 7 and 60 exposure days and ~1 ml samples of bile and blood (serum) were collected. Rats were subsequently euthanized and striatum, olfactory bulb, frontal cortex, cerebellum, liver, spleen, and femur samples collected for chemical analysis. Hematocrit was determined and was unaffected by Mn exposure. Tissue Mn and iron concentrations will be determined using inductively coupled plasma mass spectrometry (ICP-MS). Future data from ICP-MS analysis will elucidate any pharmacokinetic relationship between different routes of oral Mn exposure.

Funding sources: Afton Chemical Corporation, National Institutes of Health T35 National Research Service Award (NRSA) Short-Term Research Training Grant
IDENTIFICATION OF A CAUSATIVE VARIANT FOR DILATED CARDIOMYOPATHY IN GREAT DANES USING A WHOLE GENOME SEQUENCING APPROACH

Charles B. Jones: Veterinary Student

Kathryn M. Meurs, DVM, PhD, DACVIM: Faculty Mentor; Lhoucine Chdid, MS: Research Assistant

cbjones2@ncsu.edu; kmmeurs@ncsu.edu; lchdid@ncsu.edu

North Carolina State University College of Veterinary Medicine

Abstract:

Dilated cardiomyopathy is a primary heart muscle disorder characterized by myocardial dysfunction, cardiac arrhythmias, and congestive heart failure. Based upon pedigree analysis, an X-linked recessive mode of inheritance appears to be most likely, with age-dependent penetrance and variable clinical expression. We hypothesize that a unique genetic mutation associated with development of dilated cardiomyopathy will be identified in the Great Dane. The specific aim is through whole genome sequencing we may identify a variant causative of DCM in the Great Dane population, which may lead to genetic testing to reduce prevalence, and better long term medical management and cure.

After performing whole genome sequencing on 5 affected dogs, we compared variants that were present in at least 4/5 dogs to 41 unaffected, non-Great Danes. Variants unique to affected Danes were categorized based on cardiac significance of the genes that contained them, using scientific databases. We performed PCR and sequencing on variants of greatest cardiac significance with an additional 4 affected Danes and 4 unaffected non-Great Danes (controls). Following DNA sequencing, locations that expressed the variant in at least 3/4 affected Danes and none of the controls, underwent sample expansion to 10 affected Danes, 10 controls, and were further examined using single nucleotide polymorphism prediction tools. None of the variants were observed in all affected dogs, none of the controls, and created a problematic amino acid substitution, differing protein structure and function. Although we have ruled out genes, we continue to search for a causative mutation within our whole genome sequence approach.

Funding provided by AKC Canine Health Foundation, Inc.
Lymphoma is highly prevalent in dogs. While initial clinical responses to chemotherapy are high, the acquisition of chemoresistance is virtually inevitable. The ability to measure developing resistance during therapy would potentially allow drug substitutions and better outcomes. One way to accomplish this goal would be to measure blood levels of very small numbers of lymphoma cells, called minimal residual disease (MRD). Because each T-cell receptor (TCR) is produced by gene rearrangement and random insertion/deletion, its DNA sequence can serve as a trackable “fingerprint” of an individual T-cell clone, and be used to quantitate MRD. However, the means to measure rare circulating malignant T-cells has not been demonstrated in dogs. We hypothesized that next-generation sequencing of TCRβ rearrangements has sufficient sensitivity to detect such low numbers in a polyclonal population. This projects’ objective was to develop a PCR primer set to amplify all potential TCRβ rearrangements and assess the ability of high-throughput sequencing to quantitate malignant TCRβ burdens. A comprehensive TCRβ primer set was updated using a recent description of the genomic TCRβ locus. TCRβ cDNA libraries prepared from lymph nodes of healthy dogs and those with lymphoma were sequenced using the Illumina MiSeq platform to evaluate repertoire diversity. In T-cell lymphoma cases, the dominant TCRβ clone represented 68.0%, 18.7% and 19.0% of sequences within their respective repertoires, while no single clone exceeded 1.59% dominance in healthy individuals. Our findings demonstrate that high-throughput TCRβ sequencing has the ability to reliably identify the malignant T-cell clone in a diverse T-cell population.
ORGANIZING CHAOS – A STUDY OF THE MOLECULAR GENOMICS AND GENE EXPRESSION OF CANINE HISTIOCYTIC MALIGNANCIES

Katherine Kennedy¹  Graduate student
Jessica Durrant¹,², Alison Motsinger-Reif³,⁴, Matthew Breen¹,²,³

kakenne4@ncsu.edu, jrdurran@ncsu.edu, aamotsin@ncsu.edu, matthew_breen@ncsu.edu

Affiliations:  ¹Comparative Biomedical Science, NCSU CVM; ²Population Health and Pathobiology, NCSU CVM; ³Center for Comparative Medicine and Translational Research, NCSU CVM; ⁴Statistics, North Carolina State University

ABSTRACT

Histiocytic malignancies (HM) are rare, highly aggressive tumors with limited response to current treatment both in human and veterinary medicine. Due to their rarity, data regarding the genomic profile are also rare. This study used molecular cytogenetics to identify recurrent DNA copy number and structural changes in canine HM. Based on these data, a custom NanoString gene expression assay was developed and implemented. Immunohistochemistry (IHC) was performed to confirm qualitative increase of proteins of interest. Seventy cases of canine HM were evaluated for genome-wide copy number aberrations using array comparative genomic hybridization at 13kb resolution. Structural aberrations were assessed using fluorescence in-situ hybridization (FISH) chromosome paints. All samples presented with extensive genomic disruption, with highly recurrent deletions involving chromosomes 16 and 31. Previously reported genes with frequent deletions in the disease (PTEN, CDKN2A, RB1) were confirmed. Structurally, canine HM demonstrated a chaotic karyotype, characterized by large, multi-centromeric chromosomes. Cases demonstrated high tumor heterogeneity, with minimal clonal aberrations within and between samples. Gene expression studies showed disruptions in the mitotic spindle assembly complex including Aurora Kinases A and B (p-value <0.0001) and significant increases in genes associated with tumor aggression including MMP-9 (p-value <0.001). No significant differences were found between breeds. Finally, IHC confirmed the increase in MMP-9 compared to controls, histiocytomas and plasmacytomas (p-values 0.02, 0.0042 and 0.0002 respectively), while also demonstrating a unique punctate-staining pattern in tumor cells. Taken together, these data offer exciting opportunities for the development of novel treatments and diagnostics in the management of canine HM.

Funded by:  AKC-CHF (American Kennel Club – Canine Health Foundation) & NCSU Cancer Genomics Fund
Computed tomography (CT) scanning is the established method used to capture bone anatomy when planning complex orthopedic procedures such as custom total joint arthroplasty and hemiarthroplasty. The potential lack of resolution of CT scans decreases the accuracy of reconstructed CT image data and could negatively impact surgical planning and implant design. This lack of accuracy may increase the risk of complications for the patient because of suboptimal implant fit or accelerated wear. The aim of the current project includes assessing accuracy of CT compared to high resolution methods: micro-computed tomography (µCT) and laser-based coordinate measuring machine (CMM). Forelimbs were harvested from cats euthanized for reasons independent from this study and scanned using four imaging methods: CT (longitudinal and transverse), µCT, and CMM. Dorsopalmar (DP) and mediolateral (ML) radius of curvature, DP and ML length, and surface deviation were measured and compared statistically using CMM as the gold standard. Results of our pilot study show that CT imaging overestimates the ML and DP lengths by a mean of 2.5 and 8.3% while µCT underestimates ML and DP lengths by a mean of 1.6 and 0.8%, respectively. CT scanned models exhibited a mean deviation from the CMM surface of 66 µm and µCT models deviated by a mean of 46 µm. From our pilot study, clinical CT, even at its highest resolution, is not sufficiently accurate to map the articular surface of the feline radius in preparation for a conforming hemiarthroplasty.

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FUNCTIONAL EVALUATION OF ANTIBACTERIAL PEPTIDES EXPRESSED BY ZEBRAFISH

Amanda Kortum – DVM/PhD Candidate
Hannah Jones, Amyn Murji, and Jeffrey Yoder
ankortum@ncsu.edu; hbjones2@ncsu.edu, amynmurji@gmail.com, jayoder@ncsu.edu
Department of Molecular Biomedical Sciences and Center for Comparative Medicine and Translational Research, NCSU CVM, Raleigh, NC

The increasing rate of bacterial resistance to antimicrobial agents is becoming a worldwide threat. New ways of combating infections of all types are desperately needed in veterinary and human healthcare. Antimicrobial peptides (AMPs) are biologically active molecules of the innate immune system. They are found in a wide range of species, from plants and insects to fish and mammals. The AMP NK-lysin was first isolated from the small intestine of pig and studies indicate it has antibacterial, antifungal, and antitumor properties. Our lab recently identified five NK-lysin-like (NKL) genes encoded in the zebrafish genome. We have demonstrated their expression in immune tissue and lymphocytes of zebrafish and have cloned each of their respective transcripts. We hypothesize that each zebrafish NKL peptide possesses unique antimicrobial properties and, as a group, provide complementary immune protection. Our long term goal is to define the natural antimicrobial activities of the zebrafish NKL peptides and investigate their application to multiple facets of medicine, both veterinary and human. Using custom synthetic NKL peptides, assays will be performed to determine their effectiveness against a variety of medically important bacterial isolates including *E. coli*, *Salmonella*, and *Staphylococcus aureus* as well as several drug resistant isolates. These studies will provide a framework for identifying key sequences and structural features of NKL peptides that correlate with antimicrobial activity. Using this data, we hope to gain functional insight for engineering more effective AMPs, thus contributing to the exploration of alternative methods for combating the rising population of antibiotic resistant bacteria.

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A CRITICAL NUMBER OF INTESTINAL PROGENITOR CELLS DETERMINE VIABILITY IN CASES OF LARGE COLON VOLVULUS

Cecilia R. Kucera, Lauren W. Stranahan, Liara M. Gonzalez
College of Veterinary Medicine, North Carolina State University, Raleigh, NC

Of the causes of equine colic, large colon volvulus (LCV) cases have among the worst prognoses for survival. Previous LCV studies concluded that epithelial cell loss extending beyond 60% of the crypt of Lieberkühn correctly predicted death. This crypt region constitutes the proliferative zone where stem and progenitor cells are located. The purpose of this study was to use stem and progenitor cell protein biomarkers phospho histone 3 (PH3), proliferating cell nuclear antigen (PCNA) and sex determining region Y- box 9 (SOX9) to indicate viability and prognoses in LCV cases. It was hypothesized that a critical number of stem cells is needed for crypt viability and determining prognosis in LCV cases ≥360°. Pelvic flexure biopsies were fixed, sectioned and stained with anti-PH3, SOX9, and PCNA antibody, and positive cells were counted. Data were analyzed using receiver operator curve and univariate, multivariate logistic regression. For PH3, SOX9, and PCNA, curve areas were 0.837 (p=0.0074, n=23), 0.5714 (p=0.5893, n=24), and 0.4416 (p=1.446, n=40) respectively, and cutoff values of 2 PH3 cells per crypt (sensitivity 88.89%, specificity 78.57%), 16 SOX9 (sensitivity 57.14%, specificity 70.59%), and 69.25 PCNA (sensitivity 71.4%, specificity 35.29%) were established. From the univariate regression, LCV cases with less than 2 PH3 positive cells per crypt are 18 times more likely to die (p=0.033). A multivariate regression did not improve the model. Future inclusion of additional cases will likely improve the utility of this technique in predicting outcome of LCV cases.

Research Grant: North Carolina Horse Council

Student Support: Equine Health Program
HIGH DOSE SINGLE FRACTION RADIATION THERAPY FOR PRESUMED CARDIAC HEMANGIOSARCOMA IN CLIENT OWNED DOGS: A PILOT STUDY

Danielle LaVine, Veterinary Student, NC State, College of Veterinary Medicine, Raleigh, NC

Michael W. Nolan, Lysa Posner, Emily Griffith, Bruce Keene, Sandra Tou, Teresa DeFrancesco and Tracy L. Gieger

E-mail: delavine@ncsu.edu, mwnolan@ncsu.edu, lpposner@ncsu.edu, eghohmei@ncsu.edu, bwkeene@ncsu.edu, sptou@ncsu.edu, tdefranc@ncsu.edu, tigieger@ncsu.edu

Cardiac hemangiosarcomas (cHSA) are malignant tumors of vascular origin which typically arise on the right atrium or auricular appendage. The best reported outcomes have been achieved with a combination of surgical resection and chemotherapy. However, tumor location, surgical morbidity, prognosis and financial constraints often preclude surgery. The purpose of this study was to determine if high dose single fraction radiation therapy (SFRT) for cHSA reduced frequency of pericardial effusion or prolonged survival, as compared with historical reports. Furthermore, several potential predictive and prognostic biomarkers were evaluated. Enrollment criteria included echocardiographic evidence of a right atrial/auricular mass with hemorrhagic pericardial effusion and no evidence of pulmonary metastasis on radiographs. A single fraction of 12 Gy was delivered using conventional techniques. Blood was collected (before, 4 and 24 hours after SFRT); troponin, vascular endothelial growth factor (VEGF) and ceramide levels were quantified. The frequency of pericardial effusion (number of pericardiocenteses per 2 week period) before SFRT was compared to that after SFRT. Overall survival time was quantified. In 4/5 dogs troponin levels increased at the 4 hour timepoint, linear regression supported this trend ($R^2=0.82$). Pre- and post-SFRT plasma VEGF concentrations (n=5) were not statistically significantly different at any timepoint. Ceramide levels pending. Pericardiocentesis frequency decreased after SFRT (p=0.03). Median survival time (n=6) was 79 days (range: 35 to 136 days). After SFRT, 2/6 dogs received carboplatin chemotherapy. Post-mortem histopathology confirmed cHSA in 5/6 dogs. In this population of dogs, SFRT appeared safe, and reduced the frequency of recurrent pericardial effusion and tamponade.

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EFFECT OF HYALURONIC ACID AND 3D CULTURE ON MESENCHYMAL STEM CELL IMMUNOPHENOTYPE STABILITY.

Latanya Linton¹, Veterinary Student
Alix Berglund¹, Paul Warren², Jennifer Fowler¹, Elizabeth Harris¹, Laura Dickerson¹, Matthew Fisher², Lauren Schnabel¹
llinton@ncsu.edu, lvschnabel@ncsu.edu
¹ North Carolina State University, College of Veterinary Medicine, Raleigh, NC
² Department of Biomedical Engineering, UNC-Chapel Hill/North Carolina State University, Raleigh, NC

Musculoskeletal injuries in horses are routinely treated with autologous bone marrow-derived mesenchymal stem cells (MSCs). Allogeneic MSC use would allow for immediate treatment of acute injuries, however previous work from our laboratory has proven that Major Histocompatibility Complex (MHC) mismatched MSCs are immunogenic. Our recent work revealed that continuous TGF-β2 treatment was effective at reducing baseline MHC expression but not at preventing IFN-γ induced MHC upregulation. The purpose of this study, therefore, is to evaluate novel mechanisms for controlling and stabilizing MHC expression on MSCs using hylauronic acid (HA) and 3D culture. We hypothesize that 2D culture of MSCs with HA will be effective at reducing baseline MHC expression on MSCs and that culture of MSCs in 3D on synthetic or HA nanofibrous scaffolds will result in improved immunophenotype stability compared to 2D. MSCs will be isolated from bone marrow (n=6 horses) and cultured in 2D for one passage at which time their immunophenotype will be characterized. MSCs will then be divided into treatment groups as follows: 2D control, 2D+TGF-β2, 2D+HA, 3D synthetic control, 3D synthetic+TGF-β2, 3D synthetic+HA, and 3D HA nanofibrous scaffold. MSC groups will be cultured for 5 days and then assessed for MHC I and II expression. IFN-γ challenge experiments will also be performed and culture media analyzed for inflammatory and immunomodulatory cytokine production. We are currently in the process of selecting the optimal molecular weight HA in 2D experiments and are synthesizing the HA nanofibrous scaffolds but expect to have results in the near future.

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HEIGHTENED ANAPHYLAXIS REACTION IN FEMALE MICE IS ASSOCIATED WITH INCREASED SYNTHESIS OF MAST CELL SECRETORY GRANULE-ASSOCIATED IMMUNE MEDIATORS

Emily Mackey*, veterinary student
Susan D’Costa†, Scott Laster§ and Adam J. Moeser‡
emackey@ncsu.edu
*Comparative Biomedical Science Program, NCSU CVM, Raleigh NC
†Department of Medicine, UNC, Chapel Hill, NC
§Department of Biological Sciences, NCSU, Raleigh NC
‡Department of Large Animal Clinical Sciences, Michigan State University CVM, East Lansing, MI

Sex is a predisposing factor in the prevalence and severity of many diseases. In particular mast cell (MC)-associated diseases, including irritable bowel syndrome (IBS) and IgE-mediated allergy, are more prevalent in females. Our previous studies with murine and porcine allergy and stress models demonstrated that females exhibit more severe MC-mediated anaphylaxis and stress-induced intestinal permeability. We hypothesized that heightened disease responses in females are associated with sex-specific differences in MC reactivity. Our objective was to determine whether MCs exhibit sex-specific differences. Utilizing bone marrow-derived mast cells (BMMCs) derived from female and male mice, we demonstrated that female BMMCs exhibited greater mediator release than male BMMCs in response MC stimuli. Further experiments revealed that heightened MC granule mediator release in females was not due to increased MC degranulation signaling, but a result of female MCs increased capacity to store granule mediators. To investigate a mechanism for increased mediator content in female MCs, we performed transcriptional analysis of male and female BMMCs. Results from sequencing revealed that female MCs demonstrated higher expression of genes related to mediator synthesis and secretory granule maturation processes. Taken together, these data revealed that female MCs have increased storage of mediators, likely due to the higher expression of genes related to mediator synthesis and granule maturation factors. The findings are in line with the heightened anaphylactic response exhibited by female mice. Uncovering the mechanism responsible for sex-dependent differences in MCs could allow for the development of sex-specific and more effective treatments for MC-associated diseases.

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DETECTION OF SYNCHRONOUS PRIMARY TUMORS AND PREVIOUSLY UNDETECTED METASTASES IN 736 DOGS UNDERGOING CT SCANS FOR RADIATION TREATMENT PLANNING

Magestro, Leanne¹, House Officer
Gieger, Tracy¹
imagest@ncsu.edu, tlgieger@ncsu.edu
¹North Carolina State University, Department of Clinical Sciences, College of Veterinary Medicine, Raleigh, NC 27607

Introduction: Many patients undergoing radiation therapy (RT) require CT scans for radiation treatment planning (CT-RTP). The purpose of our study was to describe synchronous primary tumors and previously undetected metastases in dogs undergoing CT-RTP and to investigate their effect on treatment recommendations and outcome.

Methods: Medical records from 736 dogs with confirmed neoplasia undergoing CT-RTP were reviewed. All scans were reviewed by a board-certified radiologist.

Results: CT-RTP abnormalities described as probable synchronous primary tumors and previously undetected metastases that resulted in additional diagnostics or monitoring and/or affected treatment recommendations were detected in 38/736 (5%). There were 25 cases with confirmed or suspected synchronous primary neoplasms, 10 with previously undetected suspected or confirmed metastases of the primary tumor, and 3 cases had both a synchronous primary tumor and metastasis. One case had two synchronous primary neoplasms. Treatment modifications included surgery for synchronous primary tumors (N=2), additional sites of RT (N=6), and addition of chemotherapy (N=4).

Additionally, patients with local or regional lymphadenopathy as described by the CT scan were further investigated. Lymphadenopathy was identified in 352 scans; 217 lymph nodes were aspirated and 49 contained metastasis. In 82 cases, nodes not identified as abnormal on CT scan were aspirated and 5 contained metastasis.

Conclusion: Although only a small number of patients had modification of treatment plans based on incidental findings discovered during CT-RTP, these findings highlight the need for thorough evaluation of CT-RTP as findings influence recommendations to perform additional staging tests and treatment for the primary and secondary tumor(s).
CUSTOM-PRINTED DRILL GUIDES FOR CANINE CERVICAL SPINAL SURGERY

Kristen Malinak: veterinary student
Christopher Mariani¹, Ola Harrysson², Denis Marcellin-Little¹

kemalina@ncsu.edu, clmarian@ncsu.edu, harrysson@ncsu.edu, djmarcel@ncsu.edu

¹ Department of Clinical Sciences, North Carolina State University College of Veterinary Medicine; ² Department of Industrial and Systems Engineering, North Carolina State University

The use of bicortical screws and polymethylmethacrylate is a recommended surgical treatment in canine cervical spine fractures and Wobbler’s syndrome. The narrowness of the implantation corridor and the proximity of the vertebral canal and vertebral arteries make screw placement difficult. In this study, canine cadavers were used to test feasibility of production and accuracy of a custom-printed drill guide with pre-determined angles calculated for safe drilling in the implantation corridor of the last four cervical vertebrae. We hypothesized that these custom guides would allow the surgeon to create accurate drill tracts by drilling in a pre-planned location. Cervical spine segments were harvested from twelve canine cadavers and were scanned using computed tomography (CT). CT images were used to build 3D digital models of vertebrae C4-C7 using Mimics software and subsequently used as the base in creation of the guide using 3Matic software. The guides were 3D-printed by an Objet350 Connex printer using a rigid opaque photopolymer and then used to create drill tracts in the desired location on the vertebrae. The drilled vertebrae were re-scanned using the CT. Preliminary results from the first specimen suggest the need to investigate the role of the guide design and the user in optimizing accuracy. Objective analysis of the deviation of the tract produced from what was planned is ongoing. The hope is that the development of a replicable protocol for production of a custom-printed drill guide may enable surgeons to use the bicortical approach with greater accuracy, mitigating the risk of surgical complications.

Funding Source: Center for Comparative Medicine and Translational Research
EVALUATION OF ULTRASOUND AS A QUANTITATIVE TOOL FOR MEASUREMENT OF CANINE LEAN MUSCLE MASS

Candace N. Matthews, Veterinary Student,
Korinn Saker, DVM, PhD, DACVN
CNMatth2@NCSU.edu; Korinn_Saker@NCSU.edu
North Carolina State University College of Veterinary Medicine

Lean muscle mass (LMM) is a vital aspect of body structure which closely reflects total body protein stores. Changes in LMM occur during the stress of illness, trauma, growth, and performance; thus quantitating LMM loss would correlate with dietary protein needs. The current LMM assessment tool for veterinary patients is subjective and poorly defined. The purpose of this project is to provide groundwork for the validation of ultrasound (US) as a non-invasive method of quantitating changes in LMM in the veterinary hospital setting. We hypothesize that US measures will accurately reflect dissected muscle measures. Our specific aims are to compare both muscle thickness measures obtained via US and dissection; and US probe types for measurement accuracy across dogs, muscle locations, and investigators.

Canine cadavers were obtained from local shelters. Eight specific muscle sites were identified, shaved, and marked for US and dissection measure. Measurements were performed in duplicate for all locations by two separate investigators and with two different probe types.

Differences between US and dissection measures, based on muscle location, animal signalment, probe and operator will be evaluated. Significance will be set at P<0.05 level. Based on these results, correlations between US and computed tomography muscle measures will be determined on live animals to the complete validation process. Use of US to quantitate LMM will provide a noninvasive standardized technique to track total body protein and make necessary interventions to optimize patient health.

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E-type prostaglandins (PGEs) play diverse roles throughout the body, including regulation of gastrointestinal (GI) homeostasis and modulation of inflammation. To treat inflammation, anti-inflammatory drugs such as NSAIDs are designed to inhibit prostaglandin production. Unfortunately, side effects of NSAIDs include GI injury in horses. To prevent and treat NSAID-associated GI injury, equine practitioners commonly administer the PGE1 analog, misoprostol. In addition to serving as a gastroprotectant, misoprostol has been shown to have anti-inflammatory effects in cell models. At the cellular level, misoprostol acts as an E-prostanoid (EP) receptor agonist, leading to increased levels of intracellular cAMP. Elevated cAMP dampens leukocyte effector functions, including adhesion, tissue infiltration, and cytokine and superoxide production. This data suggests potential benefits of misoprostol as an anti-inflammatory therapy in horses that could avoid negative GI consequences of NSAIDs. The effects of misoprostol on equine leukocyte effector functions have yet to be determined. Therefore, we hypothesized that misoprostol inhibits equine leukocyte pro-inflammatory cytokine and superoxide production. Primary equine leukocytes were isolated from whole blood and stimulated with lipopolysaccharide (LPS) in the presence or absence of misoprostol. Misoprostol at concentrations of 1-10uM significantly inhibited TNF-α, IL-1β, and IL-6 mRNA production, as measured by qPCR. Misoprostol also led to a concentration-dependent decrease in neutrophil respiratory burst in response to granulocyte-monocyte colony-stimulating factor (GM-CSF) priming and LPS stimulation, as measured by luminol-enhanced chemiluminescence. This data indicates that misoprostol produces anti-inflammatory effects in equine leukocytes in vitro, paving the way for in vivo studies investigating misoprostol as an anti-inflammatory treatment in horses.

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THE ROLE OF APOPTOSIS-REGULATING PROTEINS BCL-2 AND BAX IN CANINE LYMPHOMA

Author: **Kristina Meichner**: house officer (kmeichn@ncsu.edu)

Co-authors: Jonathan Fogle (jefogle@ncsu.edu), Linda English (lbenglis@ncsu.edu), Steven E. Suter (sesuter@ncsu.edu)

From the Department of Population Health and Pathobiology (Meichner, Fogle, English) and the Department of Clinical Sciences (Suter), North Carolina State University, College of Veterinary Medicine, Raleigh, NC

**Background**: Immunophenotype is critical for prognosis of canine lymphoma, but the reason for the difference in biological behavior between B-cell lymphoma (BCL) and T-cell lymphoma (TCL) is largely unknown. Dysregulated apoptosis is one hallmark of tumorigenesis, and is also involved in resistance to cytotoxic therapy. Altered expression of antiapoptotic (e.g., Bcl-2) and pro-apoptotic (e.g., Bax) molecules is clinically important in several human malignancies including lymphoma, and may also be relevant in canine lymphoma. **Objectives**: We hypothesized that 1) Bcl-2/Bax expression patterns differ between lymphoma immunophenotypes, and that 2) Bcl-2/Bax ratio is correlated with outcome. **Methods**: Immunophenotype and Bcl-2/Bax expression was prospectively determined by flow cytometry in lymph node (LN) aspirates from 55 dogs with multicentric lymphoma. In addition, Bcl-2/Bax expression was assessed by flow cytometry, qRT-PCR, and western blot in LN aspirates from dogs with multicentric lymphoma compared to healthy dogs. Progression-free survival (PFS) was retrospectively evaluated in a subset of dogs with lymphoma. **Results**: Bcl-2/Bax ratio was higher in dogs with TCL compared to BCL ($p<0.0001$) and normal dogs ($p=0.0006$), respectively. There was a trend of Bcl-2/Bax ratios correlating with PFS in both BCL and TCL, respectively. **Conclusion**: Our results indicate a higher intrinsic resistance to apoptotic stimuli of canine TCL compared to BCL, which likely contributes to the less favorable prognosis following initiation of cytotoxic treatment. A larger prospective clinical study is needed to investigate if Bcl-2/Bax ratio may provide prognostic information in addition to immunophenotype for dogs with lymphoma.

The study was supported by the NCSU Immunology Program Fund.
USING CELL TRANSPLANT TO TEST THE AUTONOMY OF THE TP53 GENE

Thomas Michal: Veterinary Student
Heather Shive DVM, PhD, DACVP
tlmichal@ncsu.edu, hrshive@ncsu.edu
NCSU CVM

TP53 influences apoptosis, cell cycle arrest, and DNA repair, making it critically important in tumor suppression. Mutation of the TP53 gene impairs these cell processes and predisposes individuals to multiple types of cancer. While the above cell-autonomous functions of TP53 are well described, less is known about non-cell-autonomous influences of TP53. We are using the zebrafish model to investigate non-cell-autonomous properties of tp53. Zebrafish are a powerful tool for analyzing genetic factors that influence cancer initiation and development. Since embryonic development is external in zebrafish, embryos can be manipulated by cell transplant at the blastula stage to create chimeric embryos. We are performing blastula-to-blastula transplants between tp53-mutant and wild type zebrafish embryos to investigate how cells of different genetic background interact in vivo. Donor cells are fluorescently labeled to enable identification and tracking in recipient embryos. Fluorescently labeled donor cells are transplanted into recipient embryos, and chimeric embryos are analyzed by immunofluorescence to detect proliferating and apoptotic cell populations. In this way we can determine how recipient cells impact donor cell survival and proliferation. Since tp53-mutant cells resist apoptosis, we expect that donor tp53-mutant cells will exhibit higher levels of survival and proliferation compared to donor wild type cells (autonomous effect). We hypothesize that tp53-mutant cells will enhance the survival and proliferation of adjacent wild-type cells (non-autonomous effect), possibly in both donor and recipient settings. These studies will provide new insight into how tp53-mutant cells modulate the microenvironment to promote cancer cell survival and growth.

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CHARACTERIZATION OF BRAF MUTATIONS IN CANINE CANCERS AND EVALUATION OF MUTATION DETECTION AS A MOLECULAR DIAGNOSTICS FOR CANINE UROTHELIAL AND PROSTATIC CARCINOMA

Hiroyuki Mochizuki¹ postdoc,
Susan G. Shapiro¹, Katherine Kennedy¹, Matthew Breen¹,²,³,⁴
hmochiz@ncsu.edu, sgbowman@ncsu.edu, kakenne4@ncsu.edu, Matthew_Breen@ncsu.edu

¹Department of Molecular Biomedical Sciences, NCSU CVM, ²Center for Comparative Medicine and Translational Research, NCSU, ³Center for Human Health and the Environment, NCSU, ⁴Lineberger Comprehensive Cancer Center, University of North Carolina

Oncogenic mutations of the BRAF gene lead to constitutive activation of the MAPK pathway. The activating BRAF mutations have been found and characterized in a variety of human cancers, leading to the development of molecular diagnostics and specific inhibitors targeting mutant BRAF in human oncology. The purpose of this study was to characterize the BRAF mutation in canine cancers and evaluate the mutation detection as a molecular diagnostics for canine urothelial and prostatic carcinoma (UC and PC, respectively).

We analyzed and characterized the BRAF mutations in 669 canine cancers of various cellular origins by Sanger sequencing. We developed a droplet digital PCR (ddPCR) assay to detect the BRAF mutation orthologous to human BRAFv600E mutation. We evaluated the assay to detect the mutation in urine specimens obtained from dogs with and without UC and PC.

The BRAF mutations orthologous to human BRAFv600E mutation was found in a variety of canine cancers, with particularly high frequency in UC and PC. We developed a highly sensitive ddPCR assay that can detect the mutation of ~0.01% frequency (1 mutation/10,000 wild type sequences). The assay was able to detect the mutation in 83% (22/26) of urine specimens of canine UC and PC patients but none of 38 control urine samples.

We demonstrated that BRAF mutation is present in a variety of canine cancers and the mutation detection in urine have the potential to serve as a non-invasive diagnostic means for canine UC and PC.

This study was funded by the NCSU Cancer Genomics Fund.
LACTATE DEHYDROGENASE ISOZYMES AS BIOMARKERS FOR THE PROGRESSION OF TUBERCULOSIS INFECTION IN GUINEA PIGS

Steven Murray¹, veterinary student

Jennifer Kopanke², Wendy Williams², Angelo Izzo³, and Lon Kendall²

swmurray@ncsu.edu, jennifer.kopanke@colostate.edu, wendy.tuttle@colostate.edu, angelo.izzo@colostate.edu, lon.kendall@colostate.edu

¹NCSU CVM, Raleigh, NC
²Laboratory Animal Resources, Colorado State University, Fort Collins,
³Mycobacteria Research Laboratories, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO

Lactate dehydrogenase (LD) is found throughout the body, with some tissues producing signature variations in LD isozymes (LD1-5). Damaged cells can release LD into the blood, resulting in altered serum isozyme values that may serve as diagnostic markers. Guinea pigs are a useful model for the study of Mycobacterium tuberculosis; however, there are no known biomarkers for assessment of disease progression. Serum LD3 is known to rise in humans infected with M. tuberculosis. A similar relationship in guinea pigs could improve the researcher’s ability to determine humane endpoints that minimize animal pain and discomfort. This study investigated whether M. tuberculosis infection in guinea pigs produces LD isozyme changes that indicate infection severity. Blood was sampled from 5 guinea pigs prior to M. tuberculosis infection and 4 weeks post infection. Electrophoresis was performed on serum using Quickgel® LD kits to separate and visualize the 5 LD isozymes. Guinea pig total serum LD averaged 185 IU/L (range: 86 – 378 IU/L) pre-infection and 132 IU/L (range: 67 – 218 IU/L) post-infection. LD constituent isozymes (LD5:4:3:2:1) were 35%:22%:10%:3%:30% pre-infection and 45%:24%:9%:2%:21% post-infection. LD5 increased 12.3 +/-12.1 IU/L and LD1 decreased 16.1+/- 22.3 IU/L. Pulmonary granulomas were noted on necropsy and corresponded to an altered LD isozyme profile. This could provide a reliable diagnostic tool to gauge the progression of M. tuberculosis infections and determine terminal endpoints.

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BLOCKED DNA DEMETHYLATION OF IL2 PROMOTER INHIBITS T-REGULATORY CELL INDUCED CD8+ T CELL DYSFUNCTION IN LENTIVIRAL INFECTION.

Mukta Nag: Graduate Student

Yan Wang, Joanne Tuohy, Ravyn Njagu, Laura Edwards, Dan Bogan, Sarah Schuett, Jonathan Fogle

mnag@ncsu.edu

North Carolina State University, College of Veterinary Medicine

The quality of the CD8+ T cell response is directly related to control of acute HIV infection. For most HIV-infected individuals, virus-specific CD8+ T cell activation is followed by dysfunction, which is characterized by poor T cell proliferation and decreased production of cytokines essential to growth and maturation such as interleukin-2 (IL2). Epigenetic changes such as DNA demethylation at the IL2 promoter are essential for IL2 production. Using the feline immunodeficiency virus (FIV) model for HIV, we have reported that immunosuppressive T regulatory (Treg) cells are activated early and during the course of infection. We have also reported decreased IL2 mRNA and concurrent increased Foxp3 mRNA in virus-specific CD8+ T cells following coculture with Treg cells. Based upon these findings, we hypothesize that epigenetic changes in the IL2 promoter, while essential for IL2 production, render the CD8+ T cell highly permissive to Treg-induced, Foxp3-mediated suppression of IL2; and that blocking Foxp3 access will prevent suppression of IL2 production. This is an important question to ask, because rescuing virus-specific CD8+ T cell function is key for developing new HIV vaccine and therapeutic strategies. Here we demonstrate that blocking DNA demethylation in feline lymphocytes prior to Treg coculture results in increased IL2 mRNA compared to controls. Further, chromatin immunoprecipitation (ChIP) demonstrates a reduction in Foxp3 binding to the IL2 promoter upon blocking demethylation. These data suggest that DNA demethylation is required for successful Foxp3 binding, indicating the potential of DNA demethylation inhibitors for rescuing CD8+ T cell function during HIV infection.
CD8+ T-CELL RESPONSES AGAINST CANINE DISTEMPER VIRUS ARE RESTRICTED BY THE CLASSICAL MHC CLASS I GENE, DOG LEUKOCYTE ANTIGEN (DLA)-88

Paige Nemec¹: Graduate Student
Alex Kapatos¹, Jennifer C. Holmes¹, Peter Ross¹, Steve E. Suter¹, Paul R. Hess¹
psnemec@ncsu.edu; paul_hess@ncsu.edu
¹Dept. of Clinical Sciences, NCSU CVM

Classical Major Histocompatibility Complex class I (MHCI) molecules, present on the surface of all nucleated cells, display peptides derived from intracellular proteins to CD8⁺ T cells, and thus constitute an important surveillance mechanism against infection and malignancy. In dogs, studying CD8⁺ T-cell responses is impeded because classical MHCI genes have not been formally established. Of the four expressed MHCI genes, only Dog Leukocyte Antigen (DLA)-88 is considered classical. In indirect support of this contention, we’ve found extensive polymorphisms at this locus, and presentation of diverse self-peptides by a representative allele, DLA-88*50801. Hence, we hypothesize that DLA-88 is a classical MHCI gene. To investigate this supposition, we used canine distemper virus (CDV) as a model pathogen, and sought to determine whether viral peptides presented by DLA-88*50801 would stimulate CD8⁺ T cells from a vaccinated dog. The CDV genome encodes >19,000 possible peptides capable of binding DLA-88*50801. To reduce this list to a testable number, peptides eluted from FLAG-tagged DLA-88*50801 immunoprecipitated from >3 x 10⁹ CDV-infected canine histiocytic cells were identified by mass spectrometry. Eleven peptides from 6 CDV proteins were validated as genuine binders by peptide-MHC stabilization assay; a 12th peptide from fusion protein was predicted by the public algorithm, NetMHCPan. To examine T-cell responses, peripheral blood lymphocytes from a *50801+ dog were stimulated with *50801-expressing murine cells pulsed with pooled synthetic peptides. Interferon-gamma production (via intracellular cytokine assay) by CD8⁺ T cells in response to CDV peptides confirms presentation by DLA-88*50801. We conclude that DLA-88 is a classical MHCI gene.

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EVALUATION OF THE IMPACTS OF EPILEPSY IN DOGS ON THEIR CAREGIVERS

Julie A. Nettifee, RVT, BS, VTS (Neurology) STAFF
Emily H. Griffith, Ph.D,  Karen R. Muñana, DVM, MS, DACVIM (Neurology)
janettif@ncsu.edu, emily_griffith@ncsu.edu, karen_munana@ncsu.edu
Affiliation(s): NCSU-CVM and NCSU Department of Statistics
Funding Source: Emma’s Fund for Canine Epilepsy (a registered NCVMF fund)

Abstract

Epilepsy is a common problem in dogs, and management of this chronic disorder requires a substantial commitment on the part of the pet owner. The aim of this study was to evaluate the impact of epilepsy in dogs on their owners, utilizing an online survey tool. A questionnaire was developed to explore a variety of factors, including seizure history, treatment, outcome, quality of life, costs associated with therapy, and sources of support. Summary statistics were calculated for responses to all questions. Quality of life comparisons were performed using ANOVA, with a significance level of p<0.05. A total of 225 responses were obtained. The majority of respondents reported positive scores for overall quality of life, although scores were significant higher for dogs that experienced isolated seizures rather than clusters (p=0.043), had a lower average monthly seizure frequency (p=0.0041), were not administered additional medication during episodes of seizures (p=0.0031 and did not experience drug related adverse effects (p<0.0001). The median monthly expenditure for antiepileptic medication was $51-$75. Despite the considerable financial burden placed on the dog owner, monthly medication cost was not associated with quality of life score. Few published reports follow dogs with epilepsy after diagnosis, and there is a growing need to understand the issues associated with long-term management of this population. The results of this study provide useful information that may help veterinary professionals educate owners and set expectations regarding treatment of seizures and quality of life for dogs with epilepsy.
AN EX VIVO COMPARISON OF COMMERCIALLY AVAILABLE ORAL REHYDRATION SOLUTIONS IN WEANED PIGS

1Amanda Neujahr: DVM student
1Laura L. Edwards, 2Jeremy S. Pittman DVM, and 3Adam Moeser DVM, PhD

alneujah@ncsu.edu, lledwar3@ncsu.edu, jeremypittman@murphybrownllc.com, moeserad@cvm.msu.edu

1NC State University College of Veterinary Medicine, 2Murphy Brown LLC, 3Michigan State University College of Veterinary Medicine

Oral rehydration solutions (ORS) have been remarkably effective in reducing morbidity and mortality attributable to childhood diarrhea since their implementation in the late 1960s. Although the physiology, safety, and efficacy of ORS have been well studied and ORS have been clinically effective treating diarrheal disease in humans, investigation into these aspects of ORS marketed for animals has been limited. Field observations along with preliminary data from Ussing chambers suggest that current dosage recommendations for commercial products may be insufficient to induce water absorption to combat dehydration. The study objective was to determine whether Ussing chambers are a suitable ex vivo method for evaluating ORS products by comparing three commercial ORS products labeled for use in swine to the gold standard ORS products World Heath Organization (WHO)-ORS and Pedialyte® (Abbott). Ileum was obtained from weaned pigs (n=6) and mounted on Ussing chambers. After a 15 minute equilibration period, the ileal mucosa was exposed to the experimental ORS at three concentrations: (1) as-labeled (2) 2x label recommendation and (3) 4x label recommendations. The change in short circuit current (Isc) induced by ORS treatments was recorded as an index of apical Na+-glucose cotransporter 1 (SGLT1) activation, a central mechanism in ORS-mediated fluid absorption. Our study aims are designed to provide a foundation for future research aiding in the understanding of ORS. Ultimately, we aim to apply the knowledge gained from the ex vivo model to help veterinarians and producers use ORS most effectively to reduce the morbidity and mortality associated with diarrheal diseases that translate to economic losses suffered during the post-weaning period.

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Merial, Inc.
ENVIRONMENTAL MODIFICATIONS TO IMPROVE OLFACTORY LEARNING AND MEMORY FOR AMMONIUM NITRATE EXPLOSIVES DETECTION

Mark R. Nichols, veterinary student
Melanie L. Foster, Heather C. Brown, David C. Dorman
mrmichol@ncsu.edu
NCSU CVM

The detection of ammonium nitrate (AN) and other fertilizer-based explosives by scent detection animals can be challenging. Our objective is to determine whether postnatal scent exposure to AN will improve the ability of adult animals to identify AN-based explosives, and we hypothesize that it will. In this experiment, litters of F344 rats were randomly assigned to either control (clean bedding) or scent enrichment (with AN or rosemary) groups. Scent stimuli were continuously present in tea balls on postnatal days 1-67. We subsequently tested littermates on an olfactory memory test and a “go/no go” AN olfactory discrimination task. In the olfactory memory test, rats were conditioned to associate a novel odor (vanillin) with a buried sugar pellet. Following the conditioning phase, we concealed AN, octanol, or amyl acetate under bedding in a random location and measured the time spent investigating the odor before and after a randomly assigned time interval (30, 120, or 180 min). Over 50% of rats from each group were able to correctly discriminate AN (CS+) from amyl acetate (CS-) during the olfactory discrimination test. Rats with AN odor enrichment and rats from the control group averaged 39 days to learn the discrimination task while rats from the rosemary enrichment group averaged 49 days. Our preliminary odor memory test results indicate that rats with AN odor enrichment respond similarly to the control group. Rats with postnatal exposure to rosemary had decreased odor memory, however this effect was not statistically significant (p = 0.0863).

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A NOVEL EXTRACTION METHOD FOR THE PREPARATION OF HEPARINIZED CHICKEN (*GALLUS GALLUS DOMESTICUS*) AND HORSE (*EQUUS CABALLUS*) WHOLE BLOOD FOR $^1$H-NMR METABOLOMICS USING DRABKIN’S REAGENT

Jennifer N. Niemuth, DVM (graduate student)
Michael K. Stoskopf, DVM, PhD, DACZM
jennifer_niemuth@ncsu.edu, micheal_stoskopf@ncsu.edu
North Carolina State University, College of Natural Resources, Fisheries, Wildlife, & Conservation Biology
North Carolina State University, College of Veterinary Medicine, Department of Clinical Sciences
North Carolina State University, Environmental Medicine Consortium

Heparinized whole blood (HWB) collection requires minimal equipment, is easily accommodated in field studies, and can be a valuable resource for nuclear magnetic resonance (NMR)-based metabolomics studies where it offers a different metabolic profile than plasma. The preparation of HWB samples for NMR analysis requires sample homogeneity. Various mechanical or chemical hemolysis methods are inappropriate for NMR and metabolomics because they alter the metabolome. We examined Drabkin’s reagent (DR), a solution originally developed for the quantitative determination of hemoglobin concentration, for its potential as an effective extraction and quenching agent, which would be invisible to $^1$H-NMR. HWB samples from a chicken (*Gallus gallus domesticus*) and a horse (*Equus caballus*) were individually mixed 1:10 v:v HWB:DR. Spectrophotometric evaluation (540 nm) of DR treated samples revealed little change in absorbance after ~10-15 min. All samples thereafter were held for 10 min after DR addition. Addition of DR to a treated HWB pellet had >95% decrease in absorbance, supporting 1:10 HWB:DR as providing full extraction. DR was invisible to $^1$H-NMR. Samples frozen 30 min after treatment produced $^1$H-NMR spectra not significantly different from samples frozen 140 min after treatment (two-tailed paired-comparison random permutation test, R=1000; chicken: p=1, horse: p=0.999). DR provided an effective extraction invisible to $^1$H-NMR, while also quenching metabolism. This methodology should be considered for metabolomics investigations where HWB is the only feasible sample and/or if the research questions are specific to erythrocyte metabolites.

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MICROSCOPIC AND MOLECULAR IDENTIFICATION AND SPECIATION OF LUNGWORM INFECTIONS IN CATS IN NORTH CAROLINA

Adeline R Noger, veterinary student
James R Flowers, Henry S Marr, Stephen H Stauffer, Michael G Levy, Adam J Birkenheuer
arnoger@ncsu.edu, james_flowers@ncsu.edu, hankmarr@gmail.com, shstauff@ncsu.edu, mike_levy@ncsu.edu, adam_birkenheuer@ncsu.edu

North Carolina State University, College of Veterinary Medicine

Aelurostrongylus abstrusus and Troglostrongylus species cause pneumonia and respiratory symptoms in a variety of felid species. Aelurostrongylus infections have been reported in domestic cats (Felis catus) in the United States and Troglostrongylus infections have been reported in domestic cats in Europe and wild felids in the United States. To our knowledge there is no current information on the prevalence of lungworm infections in domestic cats in the United States. In this study we hypothesized that lungworm infections will be present in cats in North Carolina. During this study a convenience sampling of fecal specimens were collected from 50 feral cats presented to a TNR (trap, neuter, return) clinic in Wake County, North Carolina. Fecal samples were collected from anesthetized cats and a modified Baermann technique was developed and utilized in the field. A portion of each sample was also stored in ethanol in preparation for DNA extraction. Each sample undergoing the modified Baermann technique was examined via light microscopy and a larvae count was performed. DNA was extracted from the samples stored in ethanol using the Zymo Research ZR Fecal DNA MiniPrep Kit. Real time polymerase chain reaction (PCR) was performed to detect A. abstrusus and Troglostrongylus. Sample collection and diagnostics are ongoing. This study will represent the first investigation of the prevalence of lungworm infections in cats in North Carolina and may help clinicians and parasitologists to better understand the health implications of feline lungworm infections for domestic cats.

Research Grant: None
Student Support: T-35 Interdisciplinary Biomedical Research Training Program, NIH T35OD011070
PHARMACOKINETICS AND PHARMACODYNAMICS OF AN EXTENDED-RELEASE BUPRENORPHINE FORMULATION IN DOGS

Sara Ostenkamp\textsuperscript{a}: veterinary student
Kristen Messenger \textsuperscript{a}, Duncan Lascelles\textsuperscript{b}, Michele Barletta\textsuperscript{c}
kmmessen@ncsu.edu
\textsuperscript{a}North Carolina State College of Veterinary Medicine, Department of Molecular Biomedical Sciences
\textsuperscript{b}North Carolina State College of Veterinary Medicine, Department of Clinical Sciences
\textsuperscript{c}University of Georgia College of Veterinary Medicine

The options for effective and safe long-term post-operative analgesia in canine patients are very limited. The purpose of this study was to describe the pharmacokinetics and pharmacodynamics of an extended-release buprenorphine (ERB) formulation in healthy adult dogs. We hypothesized that plasma concentrations associated with therapeutic efficacy would be maintained for 72 hours after a single subcutaneous administration of ERB. Six healthy mixed breed dogs were administered either ERB (0.2 mg/kg subcutaneously) or intravenous buprenorphine (IVB, 0.02 mg/kg) in a prospective, randomized, blinded, positive control crossover study. Blood samples were collected and analyzed for plasma buprenorphine concentrations using ultra high pressure liquid chromatography/mass spectrometry. Thermal withdrawal latency, sedation scores, temperature, and heart and respiratory rates were obtained at predetermined intervals for 6 days in each study period. Maximum plasma concentrations (median, range) of buprenorphine in dogs receiving ERB was 5 (4.3-11.0) ng/mL, which occurred at 8 (4-36) hours (T\textsubscript{max}). Therapeutic plasma concentrations (>1 ng/mL) occurred from 0.5 to 72 hours after administration in most (5/6) dogs. Thermal withdrawal latencies were significantly prolonged in the ERB group compared to the IVB group from 4 to 60 hours. Sedation scores were not significantly different in dogs receiving IVB or ERB. After administration of ERB, some dogs (2/6) experienced decreased appetite and all dogs had decreased fecal output, however, no serious adverse effects were seen in either treatment group. These results suggest that a single subcutaneous dose of 0.2 mg/kg ERB is safe, provides consistent therapeutic plasma concentrations, and exhibits prolonged analgesia in healthy dogs.

Funding sources: Merial
INVESTIGATING THE GROWTH AND MORPHOLOGY OF THE ΔMSRMSD MUTANT IN SALMONELLA

Jessica Palmer: veterinary student, NCSU CVM c/o 2018
Kimberly Schreiber¹, Michelle Nauerth¹, Janessa Winston¹, Johanna Elfenbein¹
jdpalme2@ncsu.edu, kaschrei@ncsu.edu, mjnauert@ncsu.edu, jeandrze@ncsu.edu, jrelfenb@ncsu.edu
¹NCSU CVM

Salmonella is best known for the hundreds of millions of cases of gastroenteritis and hundreds of thousands of deaths it causes annually. The ability to colonize the intestine of susceptible hosts and to withstand low temperatures occurring during food processing and storage are critical for the success of this foodborne pathogen. We previously discovered that a hybrid RNA-DNA molecule called multicopy single-stranded DNA (msDNA) plays a prominent role during host infection by Salmonella. There are three necessary components for msDNA production: msr (the primer and RNA part of msDNA), msd (encoding the template for reverse transcription), and a reverse transcriptase (STM3846). I hypothesized that msr encodes the functional portion of msDNA. To test this hypothesis, I analyzed the growth and morphology of the mutants lacking this gene and compared them to the wild-type organism and Δmsd and ΔSTM3846 mutants in different environmental conditions and during murine host infection. I observed that the Δmsrmsd mutant has defective anaerobic and low temperature (15°C) growth. The kinetics of growth in these conditions differ from the Δmsd and ΔSTM3846 mutants. During infection of the murine host, the Δmsrmsd mutant shows defective growth in the large intestine compared to the ΔSTM3846 mutant. Interestingly, the morphology of the Δmsrmsd mutant appears to differ from that of other msDNA-deficient mutants and from the wild-type organism when grown aerobically. Together, these data suggest a unique role for msr in the function of msDNA. Continuing work will further define the role of msr in the function of msDNA.

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THREE-DIMENSIONAL ASSESSMENT OF THE SHAPE OF THE CANINE RADIUS USING COMPUTER AIDED DESIGN SOFTWARE

Karen M. Park\textsuperscript{1}, veterinary student
Denis J. Marcellin-Little\textsuperscript{1}, Caroline E. Webster\textsuperscript{2}, Ola L.A. Harryson\textsuperscript{2}, Amanda L. Bell\textsuperscript{3}, Sara E. Russell\textsuperscript{3}
Email addresses: kmpark2@ncsu.edu; djmarcel@ncsu.edu; cewebst2@ncsu.edu; oaharrys@ncsu.edu; albell3@ncsu.edu; serusse2@ncsu.edu

\textsuperscript{1}Department of Clinical Sciences, College of Veterinary Medicine, NCSU
\textsuperscript{2}Edward P. Fitts Department of Industrial and Systems Engineering, College of Engineering, NCSU
\textsuperscript{3}Department of Statistics, College of Agriculture and Life Sciences, NCSU

Introduction: Computer aided design (CAD) software is being increasingly used to assess bone shape and plan complex orthopedic procedures, including total joint replacement, limb sparing, and bone deformity corrections. In dogs, modeling the shape of the radius is particularly important because the radius is the most common bone for deformity correction, the most common site of limb sparing, and a common fracture site. Unfortunately, little is known about the repeatability of CAD assessment of bone shape and methods modeling the shape of the canine radius are not standardized.

Objectives: To develop and optimize a CAD process to assess the shape of normal and abnormal canine radii. Our goals were to evaluate the accuracy, repeatability, and convenience of several CAD software methods used to assess the geometry of normal and abnormal canine radii.

Methods: CT scans of normal and abnormal radii from dogs referred to the NCSU Veterinary Teaching Hospital were imported into CAD software and reconstructed. The shape of the bone was analyzed using custom-developed software as the gold standard and three different methods used within a commercially available CAD software program. Medial-lateral (ML) angulation, cranial-caudal (CC) angulation, and rotation were measured.

Results: Six normal and 11 abnormal radii were measured. Fitting best-fit cylinders to each radial bone segment should produce more accurate and repeatable measurements of the three-dimensional orientation of the three portions of the canine radius.

Conclusions: Data collected from this study will be used to plan corrective osteotomies on dogs with deformed radii using optimized planning steps.

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POPULATION PHARMACOKINETICS OF ENROFLOXACIN AND ITS METABOLITE CIPROFLOXACIN IN THE GREEN SEA URCHIN (STRONGYLOCENTROTUS DROEBACHIENSIS) FOLLOWING INTRACOELOMATIC AND IMMERSION ADMINISTRATION

Brianne E. Phillips, DVM (oral presentation): house officer
Craig A. Harms, DVM, PhD, DACZM; Gregory A. Lewbart, VMD, DACZM; Lesanna L. Lahner, DVM, MPH; Martin Haulena, DVM, MSc, DACZM; Justin F. Rosenberg, DVM; and Mark G. Papich, DVM, MS, DACVCP.

From the NCSU CVM (Phillips, Harms, Lewbart, Papich), Seattle Aquarium, Seattle, WA (Lahner), and Vancouver Aquarium, Vancouver, British Columbia, Canada (Haulena, Rosenberg).

bephill2@ncsu.edu, caharms@ncsu.edu, galewbar@ncsu.edu, l.lahner@seattleaquarium.org, martin.haulena@vanaqua.org, justin.rosenberg@vanaqua.org, mark_papich@ncsu.edu

Abstract: Sea urchin mass mortality events are due to both non-infectious and infectious etiologies, including bacterial species. Aquarium collection sea urchins, also subject to bacterial infections, could benefit from antimicrobial treatment yet pharmacokinetic studies are lacking. This study evaluated the pharmacokinetics of enrofloxacin and its active metabolite ciprofloxacin in the green sea urchin (Strongylocentrotus droebachiensis) following intracoelomic injection and medicated bath immersion. Thirty sea urchins were assigned to either the injection or immersion group. Twelve study animals and three untreated controls were utilized for each administration method: enrofloxacin 10 mg/kg intracoelomic injection or a 6 hour enrofloxacin 10 mg/L immersion. Each animal was sampled four times from 0-120 hours. Water samples were collected during immersion treatment and post-treatment time points in both groups. Drug concentrations were analyzed using high-performance liquid chromatography and pharmacokinetic parameters were determined using a nonlinear mixed effects modeling population pharmacokinetic method. Enrofloxacin concentrations fit a two-compartment model with first-order input for the intracoelomic injection group. The enrofloxacin elimination half-life (t½), peak hemolymph concentration (CMax), and area under the curve (AUC) were 38.82 hours, 90.92 µg/mL, and 1199 hr*µg/mL, respectively. Enrofloxacin modeled a one-compartment model with first-order input for the immersion treatment. The enrofloxacin t½, CMax, and AUC were 33.46 hours, 0.48 µg/mL, and 32.88 hr*µg/mL, respectively. Ciprofloxacin was detected in trace concentrations in all hemolymph samples indicating minimal production of this metabolite. The enrofloxacin concentrations achieved far exceeded minimum inhibitory concentrations reported for teleost pathogens. No adverse effects were associated with enrofloxacin administration by either treatment method.

Funding sources: Funding for the pharmacokinetic analysis was provided by the American Association of Zoo Veterinarians Wild Animal Health Fund and funding for supplies was provided by the Vancouver Aquarium.
ACETYLCHOLINESTERASE PLAYS A NON-NEURONAL, NON-ENZYMATIC FUNCTION IN INTESTINAL DEVELOPMENT

Melissa Pickett, PhD candidate
Nanette Nascone-Yoder
mapicket@ncsu.edu, nmnascon@ncsu.edu
NCSU Environmental and Molecular Toxicology Program

The vertebrate intestine develops from a simple straight tube into a long, counter-clockwise coiled organ, adapted for digestion. Malformations of this organ in humans (1:500 live births) and domesticated animals often require surgical correction and altered life-long nutritional requirements, but little is known about the etiology of such defects. Intriguingly, chemical inhibition of the enzyme, acetylcholinesterase (AChE), results in short, malrotated intestines in animal models, leading us to hypothesize that AChE is involved in gut development. While AChE is well known for its role in the degradation of the neurotransmitter, acetylcholine, we found that AChE is expressed in the non-innervated epithelial cells of the gut, suggesting that AChE may have a non-enzymatic function in gut development. Additionally, we found that knocking down AChE within the non-neuronal gut cells alone abrogates gut elongation. Cells lacking AChE display disrupted cell polarity, abnormal shape and cytoskeletal structure, and fail to rearrange to form a mature epithelium. Importantly, we were able to ameliorate these phenotypes with expression of a mutated, non-enzymatic form of AChE. These results not only suggest that AChE is required for gut development, but that non-enzymatic functions of the protein are essential for this process to occur. This work reveals a novel, previously unrecognized role for a neurotransmitter hydrolase in coordinating polarized cell rearrangement during organ development.

Funding Source: NIH - NIDDK
A METAGENOMIC APPROACH TO STUDY THE POTENTIAL PERSISTENCE AND TRANSMISSION OF ANTIMICROBIAL RESISTANCE (AMR) SALMONELLA IN SWINE FARMS

Suchawan Pornsukarom¹, PhD student, CBS-PHP
Siddhartha Thakur²
¹spornsu@ncsu.edu
²sthakur@ncsu.edu

Abstract:

The objectives of this study are to determine the antimicrobial resistome in soil and identify the role of environment in AMR Salmonella persistence and transmission in swine farms. At the different time points of manure application: day 0, 7, 14, and 21, the soil and lagoon samples were collected representing swine farms in Iowa (n=7) and North Carolina (n=5). A total of 1,200 soil samples (IA=700; NC=500) and 120 lagoon samples (IA=70; NC=50) were included. Metagenomics was used to analyze samples collected at different time points. Antimicrobial susceptibility (AST) was conducted using Sensititre® with a panel of 15 antimicrobial drugs. Pulsed field gel electrophoresis (PFGE) and metagenomics were used for genotypic characterization. Overall Salmonella prevalence was 13.3%. The prevalence in soil and lagoon were 10.9% and 37.5% respectively. NC prevalence (28.2%) was significantly higher than IA (2.7%) (p-value<0.001). Decrease in prevalence from Day0 to Day21 was observed overtime. We identified 12 serotypes with Anatum (7.4%) and Litchfield (4.0%) in IA, while Altona (7.95%), Muenster (9.1%), Typhimurium var5- (20.5%) in NC being predominant. MDR Salmonella isolates were 80.5% with the most frequent antimicrobial resistance against streptomycin (82.8%), sulfisoxazole (73.4%), and kanamycin (61.7%). According to PFGE, we detected clonal relatedness among Salmonella recovered from lagoon and soil at multiple time points with relatively close geographic proximity. Metagenomics demonstrated most abundant phyla found in soil were proportionally different and clustered based on selection pressure specific to two different geographic locations. Our study highlights the environmental reservoirs played potential roles in AMR persistence and transmission.
The FDA established tolerance levels for drugs in milk, yet it is unknown how these drugs partition into various milk fractions such as milk fat and skim milk. Although whole milk bulk tanks permit drug concentrations below the tolerance level, partitioning may result in higher concentrations in particular fractions. This accumulation may cause consumers to ingest more than the regulated FDA tolerance level, despite the restriction placed on bulk tanks. We hypothesized that the partitioning would follow the linear regression relationship between literature log octanol-water partition coefficient (logKow) and the drug’s molecular weight; due to the nature of each drug’s hydrophilicity or hydrophobicity. The partitioning of fourteen 14C and 3H-radiolabeled drugs (logKows ranging from -0.4-6.5), were investigated in whole milk. To imitate dairy industry’s whole milk processing: we utilized raw milk, mimicked cold centrifugation, and used drugs both approved and non-approved by the FDA to provide a wide spectrum of chemical properties. The objective of this study was to understand partitioning and the roles of temperature and drug-to-contact time. We presumed no difference would show between frozen and refrigerated milk. However, frozen milk samples always indicated a higher logK [Milk Fat/Skim] (logKmfs); and longer periods were required for stabilization. The results found both in frozen (logKmfs of -1.38-0.97) and refrigerated milk (-0.94-0.14) correlated highly to the trend set by the literature logKow as predicted. This correlation can be used to produce a predictive model of drug partitioning. These results are significant in understanding drug consumption from dairy products.
THE IMPORTANCE OF BREAKOVER DISTANCE IN EQUINE HIND FEET

Megan Radkin: veterinary student
Richard Mansmann, VMD, PhD, hon. DACVIM-LA, Anthony T. Blikslager, DVM, PhD, DACVS.
mdradkin@ncsu.edu
dickmansmann@gmail.com
Affiliation: NCSU CVM

Abstract:
Breakover distance (BD) of the horse’s hind foot has been suggested as an important factor in a biomechanically normal gait as well as select causes of lameness. For example, there may be a relationship between longer BD and palpable pain in the gluteal/hip regions which can lead to decreased performance and/or behavioral problems. The objective is to examine the suggestion that shortening BD might alleviate pain, and to show that overly shortening BD might re-introduce that pain. From July 2010 to July 2015, the Equine Podiatry and Rehabilitation Mobile Practice has seen 122/364 horses that were determined, radiographically, to have “long-toed, low-heeled” (LT/LH) hind foot conformation. Of those, 89% had palpable pain in the gluteal region and their average BD was 25.84 ± 0.822mm. The BD is measured on a lateral radiograph between the distal phalanx and the most dorsal point of the wall/shoe contacting the ground. After BD was reduced to an estimated 5 to 15mm, 93.98% had improved gluteal pain and/or owner noted overall improvement. BD was determined to be too short via painful gluteal palpation in 11% (mean, 5.25 ± 1.915mm) suggesting an ideal BD range for each horse may be needed. Two future areas of investigation are 1) validating the BD on the horse versus that on a lateral radiograph, and 2) objectively measuring pain in the gluteal region with an algometer in an equine breakover model.
INVESTIGATING THE FUNCTION OF A HIGHLY CONSERVED GENE, TMEM150A

Jessica L. Romanet: Graduate Student
Jeffrey Yoder
jromanet@gmail.com; jayoder@ncsu.edu
Comparative Biomedical Sciences, Dept of Molecular Biological Sciences and Center for Comparative Medicine and Translational Research, NCSU CVM

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Cytokines are small, secreted proteins that play a critical role in cellular communication during immune responses. During an inflammatory event, cytokines trigger signaling mechanisms that result in activation of immune cells and release of free radicals, nitric oxide, and antimicrobial contents. The culmination of events is the removal of the insulting source, such as a pathogen, and initiation of tissue healing. While tight regulation of cytokine activation is vital to host survival and control of inflammation, these cytokine responses often become unchecked in a wide range of illnesses including septic shock, cancer growth, and reperfusion injury. Thus, understanding factors that control regulation of cytokine release is critical to understanding the cellular biology of the immune response, and also vital in determining new therapeutic targets to control unchecked cytokine production in a wide range of diseases.

One of the most well-defined pathways regulating cytokine production are those initiated when bacterial pathogen associated molecular patterns (PAMPs) are recognized by pathogen recognition receptors (PRRs) on immune, epithelial and fibroblast cells. Of these PRRs, Toll-like receptor 4 (TLR4) is a potent and well-studied receptor that responds to lipopolysaccharide (LPS) found in gram-negative bacterial cell walls.

Using a human cell culture model that isolates the LPS-TLR4 response, we have knocked-down a novel, highly conserved, and functionally undefined gene—TMEM150a—and have discovered increased levels of LPS-induced IL-8 release in TMEM150a deficient cells. We hypothesize, that TMEM150a, plays an essential role in signaling events that lead to cytokine release and is a potential target for therapeutic intervention.
EVALUATION OF DNA VACCINATION FOR PREVENTION OF CYTAUXZOOON FELIS INFECTION AND/OR CYTAUXZOOONOSIS IN DOMESTIC CATS: A PILOT STUDY

Megan E. Schreeg¹: Graduate Student (medowney@ncsu.edu)
Henry S. Marr¹, Jaime L. Tarigo¹,², Meredith K. Sherrill³, Hilton K. Outi³, Elizabeth H. Scholl⁴, David M. Bird⁴, Adam Vigil⁵, Chris Hung⁵, Rie Nakajima⁵, Li Liang⁵, Jennifer E. Thomas⁶, Michael G. Levy¹, Mason V. Reichard⁶, Philip L. Felgner⁵, Leah A. Cohn³, Adam J. Birkenheuer¹
1. North Carolina State University, College of Veterinary Medicine, Raleigh, NC
2. University of Georgia, College of Veterinary Medicine, Athens, GA
3. University of Missouri, College of Veterinary Medicine, Columbia, MO
4. North Carolina State University, College of Agriculture and Life Sciences, Raleigh, NC
5. University of California Irvine, School of Medicine, Irvine, CA
6. Oklahoma State University, College of Veterinary Medicine, Stillwater, OK

Cytauxzoonosis is an emerging disease of felids caused by the tick-transmitted apicomplexan parasite *Cytauxzoon felis*, an organism spreading across the United States. Cytauxzoonosis is particularly virulent for domestic cats, and most untreated cats that present to veterinary hospitals succumb to disease. The best treatment available (atovaquone and azithromycin) only confers a 60% survival rate and in conjunction with supportive care can cost thousands of dollars, limiting its widespread use. Disease prevention is currently limited to indoor confinement and prophylactic acaricides, but immunization against *C. felis* would be a more cost-effective and pragmatic approach to preventing cytauxzoonosis. Because the parasite currently cannot be cultured in vitro, we have searched the *C. felis* genome and identified 33 genes encoding candidate immunogens from which we have designed two DNA vaccines: an expression library vaccine including all candidates (CF-Library) and a vaccine containing the most well-described candidate (CF-1). We hypothesized that these vaccines could protect against infection and/or cytauxzoonosis, and tested this hypothesis in a non-randomized, partially blinded pilot study. Regrettably, all vaccinated cats became infected and developed cytauxzoonosis. However, cats vaccinated with CF-Library had the highest survival rate (100%), and one cat in this group demonstrated a comprehensive serological response to vaccination and required no therapeutic intervention. Serological response to vaccination varied highly between cats, suggesting that antigens were not uniformly delivered and/or expressed. Consequently, although neither vaccine as currently designed is recommended for further development, investigation into the utility of these antigens in a different vaccine platform is warranted.

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EVALUATION OF THE FUNCTIONAL REGION(S) OF MULTICOPY SINGLE-STRANDED DNA IN SALMONELLA

Kimberly Schreiber: veterinary student
Jessica Palmer, Michelle Nauerth, Jenessa Winston, Johanna Elfenbein
kaschrei@ncsu.edu, jdpalme2@ncsu.edu, mjnauert@ncsu.edu, jeandrze@ncsu.edu, jrelfenb@ncsu.edu
NCSU CVM

Salmonella enterica is a serious public health concern, causing millions of cases of gastroenteritis in people annually. Despite being a heavily studied pathogen, we have yet to fully understand the keys to its survival in the intestine of natural hosts. Prior work in our laboratory suggests that an essential aspect to the viability of Salmonella in the gut is tied to multicopy single-stranded DNA (msDNA), a RNA-DNA hybrid produced by the action of a reverse transcriptase. I hypothesized that the DNA portion of msDNA, composed of a 29 base-pair stem and a 4-nucleotide loop, is needed for its function. I generated targeted mutations to each of the stem and loop portions of msDNA and show that msDNA is truncated when deletions are made in the stem. In vitro during anaerobic growth, mutants with deletions in the stem have phenotypes similar to mutants lacking msDNA altogether. However, mutations in the nucleotides comprising the loop have no effect on function of msDNA during anaerobic growth or during infection in the murine host. Our data suggest that the unpaired nucleotides in the DNA loop are dispensable for the function of the molecule but that the DNA stem is either required for production of msDNA or for its stability. This supports our hypothesis that the stem portion is required for a stable and functional molecule. Further investigation will determine whether the DNA stem is required for production of msDNA or for its stability.

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Viral myocarditis (cardiac damage and inflammation) is indicated as the second-leading cause of sudden death in young adults. Cardiac myocytes (muscle cells) are poorly replenished yet are indispensable for cardiac function. Previously our laboratory reported that primary cultures of cardiac myocytes express higher basal levels of the protective cytokine interferon-β (IFN-β) than do cardiac fibroblasts or skeletal muscle cells, providing a “pre-arming” mechanism that protects them against viral infection. Here we investigated the mechanism for cardiac myocyte expression of abundant basal IFN-β. In most cells, IFN-β is expressed at low levels. Viral infection is recognized by cytoplasmic sensors that are then translocated to mitochondria and mitochondrial-associated endoplasmic reticulum membranes (MAM) where they interact with the mitochondrial-antiviral signaling (MAVS) adapter protein. MAVS then recruits enzymes to activate transcription factors resulting in induction of IFN-β. We hypothesized that cardiac muscle cells profuse with mitochondria might express high levels of MAVS resulting in leaky activation of the pathway, but that there must also be other elements that differ between cardiac and skeletal muscle cells. We found that while cardiac and skeletal muscle cells express higher basal levels of MAVS than do cardiac fibroblasts, only cardiac myocytes display spontaneous high association of MAVS with the MAM. Moreover, downstream target enzymes are spontaneously activated only in cardiac myocytes, and this activation is dependent on the presence of MAVS but not upstream cytoplasmic sensors. Together, our results suggest that abundant MAVS and association with the MAM in cardiac myocytes triggers spontaneous high basal expression of IFN-β.

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PRIAPISM IN A THOROUGHBRED GELDING ASSOCIATED WITH METASTATIC S. EQUI INFECTION

Author Name, Category: Kristina Simmons, Veterinary Student

Co-Authors: E.A. Coffman, T.M. Beachler, K. McKelvey, B. Breuhaus, C.S. Bailey

Email Address: kmsimmo2@ncsu.edu

Affiliation: NCSU CVM

A 12-year-old Thoroughbred gelding presented to the NCSU CVM for acute priapism and weight loss. Physical exam revealed poor body condition (BCS 2/9) and complete protrusion of the erect penis. The penis was reduced into the prepuce following irrigation of the corpus cavernosum with heparinized saline, injection of 10 mg of phenylephrine into the corpus cavernosum, and manual massage using nitrofurazone and DMSO ointment. A purse string suture and penile sling were placed to assist in penile retention and support. The erection recurred approximately 8 hours after the procedure, and treatment was repeated. Diagnostic procedures to pursue the weight loss included thoracic and abdominal ultrasound, complete blood count and chemistry, urinalysis, urine culture, rectal biopsy, and Streptococcus equi ELISA. Urinalysis revealed 2+ hematuria and pyuria. The serum ELISA assay for S. equi returned strongly positive (1:25,600), consistent with metastatic strangles abscesses. Treatment with trimethoprim sulfamethoxazole (TMS, 30 mg/kg PO BID) and rifampin (5 mg/kg PO BID) was instituted for 8 weeks based on evidence that rifampin may prevent resistance and improve efficacy of the TMS against Streptococcus spp. sequestered in abscesses.1 At recheck examination seventeen weeks after discharge, the gelding’s BCS was 5/9 and his priapism had improved, although the penis still protruded from the prepuce two inches at most times. Thirty weeks after discharge the animal had returned to a normal weight and the priapism was completely resolved. Priapism is rare in horses, especially geldings, and there are no reports of priapism secondary to metastatic strangles.

Reference:

AN IMPROVED LARGE ANIMAL MODEL FOR THE STUDY OF ADULT STEM CELLS

Sean G Simpson¹,² (Staff)

Liara M Gonzalez³, Jaewook Chung¹,², Anthony Blikslager³, Scott Magness⁴,⁵,⁶ and Jorge A Piedrahita¹,² (japiedra@ncsu.edu)

¹Department of Molecular Biomedical Sciences, ²Center for Comparative Medicine and Translational Research, ³Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA. ⁴Department of Medicine, ⁵Cell Biology and Physiology, ⁶UNC/NCSU Biomedical Engineering, University of North Carolina, Chapel Hill, North Carolina USA.

Mouse models for the study of adult stem cells have broadened the understanding of previously uncharacterized stem cell niches. Murine reporter lines for the LGR5 gene have highlighted its importance as a stem cell marker in the intestine, hair follicle, liver and kidney in mice. These models have significant limitations in comparative medicine due to anatomical and physiological differences between humans and mice. To overcome these limitations, we have sought to develop a porcine LGR5 reporter line.

To generate this line we used a combination of transcription activator-like effector nucleases (TALENS) and somatic cell nuclear transfer (SCNT). TALEN mediated homologous recombination was used to drive the integration the green fluorescent protein into the 3’ un-translated region of the LGR5 locus in porcine fetal fibroblast cells. Upon screening and confirmation of proper integration by DNA sequencing, SCNT using these cells was performed. Transfer of the SCNT embryos to a surrogate gilt resulted in three live births, establishing a founder line of LGR5-GFP reporter pigs.

To characterize this line, we have observed fluorescent labeling of putative stem cell populations in the intestinal crypts and hair follicles from these animals. These observations parallel the expression patterns observed in similar murine models. We have confirmed the fluorescent reporter signal by immunohistochemistry using an anti-GFP antibody.

This line represents significant progress toward the study of adult stem cells, and the stem-cell niche, using a large animal model with an anatomy, physiology, and ability to recapitulate human disease that overcomes the current limitations of rodent models.

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THE TUMOR SUPPRESSOR p53 IS A SUSCEPTIBILITY FACTOR FOR GRANULOMA FORMATION IN THE LUNGS OF MICE EXPOSED TO MULTI-WALLED CARBON NANOTUBES

Elizabeth A. Smith, Veterinary Student

Katherine S. Duke, Elizabeth A. Thompson, Mark D. Ihrie, Kelly A. Shipkowski, Alexia J. Taylor, James C. Bonner

Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695
College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27607

Nanotechnology has benefits in engineering, electronics, and human or veterinary medicine. However, some engineered nanoparticles have toxic effects. The purpose of this study was to evaluate pulmonary disease caused by multi-walled carbon nanotubes (MWCNTs), a prototypical engineered nanoparticle. We hypothesized that mice lacking one allele of the tumor suppressor gene p53 would be susceptible to cancer (mesothelioma) or non-cancer lung disease (fibrosis or granuloma) after chronic exposure to MWCNTs. We exposed mice to 4 doses of 1 mg/kg MWCNT for 1 month by oropharyngeal aspiration. After 9 months we performed quantitative morphometry on lung sections under light microscopy for fibrotic granulomas, inducible bronchus-associated lymphoid tissue (iBALT), and/or mesothelioma. Bromodeoxyuridine (BrdU) immunostaining was performed to assess cell proliferation in lung tissue. To quantify granulomas and iBALT, Adobe Photoshop software was used to measure area and perimeter and to derive the area to perimeter ratio for each lesion. Graphpad Prism software was used to perform statistical analysis. No evidence of mesothelioma was found in wild type or p53 heterozygous (p53 +/-) mice. MWCNTs caused a significant increase in granuloma size between wild type and p53 +/- mice (p<0.05). There were no differences in number or size of iBALT tissue between treatments or genotypes. Cell proliferation was observed within iBALT tissue. These findings indicate that p53 +/- mice are susceptible to pulmonary fibrosis and granuloma formation after chronic exposure to MWCNTs, yet p53 +/- mice do not develop mesothelioma after pulmonary exposure. This study has important implications for understanding the human health risks of emerging nanotechnologies.

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Student Support: Veterinary Scholars Program, NCSU
Field of Research: Pharmacology/Toxicology
Listeria monocytogenes is a rod shaped gram-positive bacterial pathogen that causes listeriosis. We are interested in how cell surface features influence the virulence of listeriae. We have constructed unmarked deletion mutations in four *L. monocytogenes* (4nonb) genes (*galU*, *gtcA*, *glcV* and *pmpA*) whose products are required for phage binding. Cell fractionation of each mutant and competition studies traced the phage binding defect directly to alterations in wall teichoic acid (WTA) composition. WTA structural (NMR) studies showed that mutant WTA lacked appreciable galactose in all but the *gtcA* mutant, which retained a galactosylation level that was ca. 7% of the parental strain. We systematically examined the requirement for WTA galactosylation for virulence *in vivo* and *in vitro*. All mutants were severely attenuated in a mouse oral-infection model, and all produced minute plaques on mouse enterocyte monolayers relative to the parental plaques. Nevertheless, all mutants bound to, and formed plaques on the enterocyte monolayers with an efficiency similar to the parental strain, properties associated with a defect in cell-to-cell spread. Microscopic examination of monolayer plaque morphology supported this conclusion. Confocal studies of intracellular bacteria revealed that the mutants failed to properly polymerize host actin on their cell surface, a step required to generate the cytosolic motility needed for cell-to-cell spread. The enzymatic steps involved in WTA galactosylation may provide attractive targets for the development of therapeutic antivirulence compounds.

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GENERATION OF STABLE LARGE ANIMAL SWINE MODEL VIA CRISPR-CAS9-MEDIATED RECOMBINATION FOR CELL TRACKING AND IN VIVO CHROMOSOME DYNAMIC STUDIES

Renan B. Sper (graduate student)
Sean Simpson, Xia Zhang, Bruce Collins, Jorge A. Piedrahita
renan_sper@ncsu.edu, jorge_piedrahita@ncsu.edu
NCSU CVM

Over the last few years transgenic pigs have become an attractive research model in the field of translational research, regenerative medicine, and stem cell therapy due to their anatomic, genetic and physiological similarities with humans. The development of transgenic murine model via fusion of GFP to Histone 2B protein (H2B, protein of nucleosome core) allows easier and convenient methods of tracking cell migration and engraftment levels after transplantation and opportunity to better understand the complexity of chromosome dynamics. Up to date the development of a stable transgenic swine model expressing H2B-GFP has not been described.

Here we describe the development of the first viable transgenic swine model expressing H2B-GFP fused β actin promoter via CRISPR-CAS9 driven homologous recombination and Somatic Cell Nuclear Transfer (SCNT). Porcine fetal fibroblast were cotransfected with custom designed CRISPR-CAS9 (targeting 3’ UTR) and targeting vector containing 1Kb homology arms flanking a IRES-H2B-GFP transgene. Four days post-transfection GFP + cells were Fluorescence Activated Cell Sorted (2.4% knock in efficiency). Single cell colonies were generated and analyzed by PCR and a heterozygous colonies used as donor cell for SCNT, resulting in 3 viable boars. Boars are currently 15 months old, express H2B-GFP ubiquitously (tissue sections and primary cultured stem cells) and were capable of generating F1 generation via artificial insemination, with a transgene transmission rates on 55.8% (in concordance with Mendel's Law). This novel large animal model represents a fundamental platform for regenerative medicine and chromosome dynamic/cancer biology studies.

Funding source: NIH R01HL051587 to JAP.
Increased awareness of antimicrobial resistant bacteria has led to the promotion of antimicrobial stewardship and judicious antimicrobial use in both human and veterinary medicine. Veterinary teaching hospitals provide a unique environment to influence graduate and postgraduate clinical veterinary education and opinions about antimicrobial use and antimicrobial resistance. Our objective was to determine opinions of faculty members with clinical appointments, clinical veterinarians, residents, and interns at a U.S. veterinary teaching hospital regarding antimicrobial use and antimicrobial-resistant infections.

An online survey was distributed to 167 clinicians and house officers at the NC State VH. The survey was open for three weeks beginning in May 2014. Survey questions focused on demographic information, factors and sources of information that influence antibiotic usage, and concerns regarding antibiotic resistance. Additionally, prescription data from 8 quarters between 2012 and 2014 were analyzed to determine the total number of antimicrobial prescriptions at the VH.

The survey was completed by 71 (43%) eligible veterinarians. Fifty-nine percent of participants classified themselves as very concerned about antimicrobial-resistant infections, while their colleagues and clients were considered less concerned. Additionally, 88% of respondents felt some or all antimicrobials were over-prescribed at VH, and 46% indicated there was an antimicrobial they were uncomfortable prescribing due to public health concerns.

Our findings indicated that veterinarians at this teaching hospital were concerned about antimicrobial resistance, thought antimicrobials were over-prescribed, and supported restricting use of certain antimicrobial classes in companion animals. These findings may be useful in educating future veterinarians and altering prescribing habits in veterinary hospitals.

Funding Sources: NCSU CVM Intramural Funds
The basis of this study was to determine the viability of zebrafish as candidates for xenotransplantation of canine cancer cells. The following canine cancer cell lines were assessed: MBSa (neurofibrosarcoma), CML-13 (malignant melanoma), TCC (transitional cell carcinoma), and UWKOS3 (osteosarcoma). Two human cancer cell lines that have been successfully xenotransplanted in previous studies were used as positive controls, HT1080 (fibrosarcoma) and SW620 (colorectal carcinoma).

The various cancer cell lines were cultured in vitro and verified via PCR to be free of any contaminants. The cells were then transfected with green fluorescent protein plasmid using Fugene Reagent. This GFP plasmid allows the transplanted cells to be visualized and assessed, both qualitatively using fluorescent confocal microscopy and quantitatively using flow cytometry. Zebrafish embryos were collected via natural mating. At 48 hours post fertilization, the transfected cancer cells were microinjected into the Duct of Cuvier, microinjecting ~500 cells per embryo. The xenotransplanted embryos were maintained and assessed with fluorescent confocal microscopy every 24 hours for a total of 72 hours. The embryos were then euthanized at 120 hours post fertilization. These embryos were processed and analyzed using flow cytometry. There was no evidence to support that any of the cancer cell lines assessed were successfully transplanted, due to a variety of complicating factors. Identifying and appropriately addressing these factors may lead to success in future studies.
TOPICALLY APPLIED MANGANESE-PORPHYRINS BMX-010 AND BMX-001 DISPLAY A SIGNIFICANT ANTI-INFLAMMATORY RESPONSE IN A MOUSE MODEL OF ALLERGIC DERMATITIS

Kelsey Stover, veterinary student
Tomoki Fukuyama, Wolfgang Bäumer
kestover@ncsu.edu, tfukuya@ncsu.edu, wbaeumer@ncsu.edu
Department of Molecular Biomedical Sciences, NCSU CVM

BACKGROUND: There is a need to develop more effective therapeutic agents for alleviation of symptoms of allergic skin disorders. In this study, we topically administered two antioxidant compounds, which are manganese-porphyrin-derivatives BMX-010 and BMX-001, in a mouse model of allergic dermatitis and compared the efficacy for reduction of itch and inflammation.

METHODS: Ear Swelling Test: Cream preparations of BMX-010 and BMX-001 (doses 0.05%, 0.1%), a vehicle (cream only), and a positive control (triamcinolone acetonide 0.015%) were topically applied 16 h and 30 min pre-challenge of TDI (7 mice in each group). Post-treatment ear thickness was measured 24 h after TDI challenge and compared to basal values. The mice were sacrificed and the ear auricle and auricular lymph node were removed for further analysis (histology, cytokine production, lymph node weight, cell counts). Assessment of Scratching Behavior: Cream preparations of vehicle, BMX-010, and BMX-001 (doses 0.1%) were topically applied 16 h and 30 min before TDI challenge, which was followed by 1 h video monitoring. RESULTS: Mice treated with BMX-010 and BMX-001 showed a dose dependent decrease in ear thickness but no reduction in scratching behavior. IL-1β and IL-4 production in the ear skin were significantly decreased, as well as lymph node weight and cell counts in the BMX treatment groups compared to the vehicle. However, the positive control group (triamcinolone acetonide) inhibited the inflammatory response to a greater extent. CONCLUSION: These first results suggest the potential use of a BMX-010 and BMX-001 cream as a supplement in treating allergic-inflammatory skin diseases.

Funding Sources:
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EFFECTS OF MATRIX METALLOPROTEINASES ON THE PERFORMANCE OF PLATELET FIBRIN GEL SPIKED WITH CARDIAC STEM CELLS IN HEART REPAIR

Authors Name: Junnan Tang  Graduate Student
Co-Authors: Tianxia Zhang, Deliang Shen, Michael Taylor Hensley, Taosheng Li, Thomas George Caranasos, Jinying Zhang, Ke Cheng
E-mail address: jtang8@ncsu.edu
Affiliations: 1. Department of Molecular Biomedical Sciences and Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University; 2. Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University; 3. Department of Cardiology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China; 4. Department of Stem Cell Biology, Atomic Bomb Disease Institute, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan; 5. Division of Cardiothoracic Surgery, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA.

Abstract:

Background: Cardiac stem cells (CSCs) have been proven to be safety and effective in treating myocardial infraction (MI). We aimed to explore the therapeutic benefits of intramyocardial injection of CellGel (i.e. platelet fibrin gel spiked with CSCs) in a rat model of acute MI with or without MMP inhibitor GM6001 (Figure 1).

Methods: CSCs were embedded in the platelet fibrin scaffold during gel formation and then formed CellGel. Rats were randomized into four groups: 1) MI + Control group: injection of vehicle (DMEM); 2) MI + CellGel group: injection of platelet fibrin gel spiked with CSCs; 3) MI + CellGel + GM6001: injection of platelet fibrin gel spiked with CSCs plus daily intraperitoneal injection of GM6001; 4) MI + CellGel+ GM6001 NC: The same as Group 3) with the use of GM6001 Negative Control.

Results: In vitro, MMP inhibitor GM6001 decreased cell body elongation and cardiomyocyte contraction. In vivo, the MMP inhibitor blunted the recruitment of endogenous cardiovascular cells into injected biomaterials therefore hindering de novo angiogenesis and/or cardiomyogenesis. Echocardiography revealed MMP inhibition diminished the functional benefits of CellGel in rats with MI. Reduction of cell engraftment, host angiogenesis, and endogenous cardiomyocyte cycling were evident in animals treated with MMP inhibitor GM6001, suggesting MMPs are essential for the indirect regeneration pathways of CellGel.

Conclusions: MMP plays a critical role in the therapeutic benefits of platelet fibrin gel spiked with cardiac stem cells for treating myocardial infarction.

Figure 1. Schematic of overall study design.
OSTEOSARCOMA INDUCES IMMUNOSUPPRESSION BY INHIBITING MONOCYTE CHEMOTAXIS AND PROMOTING T-REGULATORY CELLS

Joanne Tuohy (graduate student)
Ravyn Njagu, Yan Wang, Mukta Nag, Duncan Lascelles, Jonathan Fogle
joanne_tuohy@ncsu.edu; rsnjagu@ncsu.edu; ywang102@ncsu.edu; mnag@ncsu.edu; duncan_lascelles@ncsu.edu; jonathan_fogle@ncsu.edu

North Carolina State University College of Veterinary Medicine, Raleigh, NC

Osteosarcoma (OSA), a devastating primary bone tumor of dogs and humans, has not seen improvement in survival in over 20 years. Mechanistic understanding of tumor-induced immunosuppression in OSA informs immunotherapy development to improve OSA outcomes. We previously demonstrated that monocyte chemokine receptors are downregulated and monocyte PGE$_2$ levels are higher in OSA dogs. PGE$_2$ is known to suppress monocyte chemotaxis and T-cell responses. Hence we hypothesize that OSA evades the immune response by inhibiting monocyte chemotaxis and inducing T-helper (Th) cells to adopt an immunosuppressive T-regulatory (Treg) phenotype (CD25$^+$Foxp3$^+$) and function. Our objectives were i) to compare monocyte chemotaxis between OSA and healthy dogs, ii) to compare Foxp3/CD25 expression and Foxp3 mRNA levels between Th cells cultured alone or co-cultured with OSA cells, iii) to compare Th proliferation when cultured alone or co-cultured with OSA-induced Treg cells. Monocyte chemotaxis was assessed using an in vitro cell migration system. Flow cytometry was used to compare CD25 and Foxp3 expression, and Th cell proliferation. qPCR used to assess Foxp3 mRNA. We demonstrate decreased monocyte chemotaxis in OSA dogs, increased intracellular Foxp3 and Foxp3 mRNA expression in Th cells following co-culture with OSA cells, and decreased Th cell proliferation when co-cultured with OSA-induced Treg cells. Collectively, these data suggest that OSA-induced immunosuppression impairs monocyte chemotaxis and converts Th cells to Treg cells, which, in turn, suppress Th cells. These findings are pivotal to understanding how OSA escapes immune recognition as monocytes and Th cells are central to coordinating the anti-tumor immune response.

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PCR PREVALENCE OF TICK-BORNE ORGANISMS IN NICARAGUAN HORSES

Jeffrey D. Tyrrell, pre-veterinary student

Co-Authors: Barbara A. Qurollo, Barbara C. Hegarty, Julie Bradley, Susan J. Tornquist, Kathryn G. Schlaich, Jennifer Kelsey, Edward B. Breitschwerdt

Email addresses: jdtyrrel@ncsu.edu, baguroll@ncsu.edu, bhegarty@ncsu.edu, julie_bradley@ncsu.edu, susan.tornquist@oregonstate.edu, schlaick@onid.oregonstate.edu, ebbreits@ncsu.edu

Affiliations: North Carolina State University, College of Veterinary Medicine, Raleigh, NC (J.D. Tyrrell, B.A. Qurollo, B.C. Hegarty, J. Bradley, E.B. Breitschwerdt), Oregon State University, College of Veterinary Medicine, Corvallis, OR (S.J. Tornquist, K.G. Schlaich, J. Kelsey)

Abstract: Tick-borne disease has significant impact on the health and welfare of horses. In order to assess the prevalence of equine tick-borne illness in the region, blood samples were collected from 93 horses in Mérida, Nicaragua. DNA was extracted from whole blood, and qPCR assays were performed to test samples for infection by bacterial species within the following genera: Anaplasma, Babesia, Bartonella, Ehrlichia, Mycoplasma, Rickettsia and Theileria. For samples with positive results, the infectious agent was speciated by species-specific qPCR assay of the sample and/or DNA sequencing of the PCR amplicon. Total prevalence for tick-borne illness was 24 out of 93 horses (25.8%). Of the horses tested, 9 (9.7%) were positive for infection by a novel Ehrlichia species recently detected in horses. Eighteen samples (19.4%) contained DNA from Babesia caballi, and 19 samples (20.4%) contained DNA from Theileria equi. Coinfection with B. caballi and T. equi occurred in 17 horses (18.3%), five of which were also infected with the novel Ehrlichia species. None of the samples contained DNA from Anaplasma, Bartonella, or Mycoplasma species. Two samples (2.2%) were positive for infection by Rickettsia felis, constituting the first reported instances of equine infection with R. felis detected by PCR. One of the R. felis-infected horses was coinfectected with B. caballi and T. equi.

Funding Sources: Oregon State University, College of Veterinary Medicine and NCSU-VBDDL
**In Vitro and In Vivo Correlation of Sustained Release IgG from a Biocompatible ThermoSensitive Polymer for Ocular Drug Delivery**

Mary Walsh, Veterinary Student  
Beth Salmon, Kaitlyn Walsh, Brian Gilger

mlwalsh@ncsu.edu, bgilger@ncsu.edu

Affiliation: North Carolina State University, College of Veterinary Medicine

**Introduction:** Ocular barriers prevent drugs from penetrating into the eye requiring frequent topical applications to reach therapeutic drug levels. Slow release, biodegradable drug delivery systems that achieve sustained therapeutic concentrations would be a major advancement. The purpose of this study was to compare in vitro and in vivo release of a model therapeutic protein from novel biocompatible gels. We hypothesized that in vitro and in vivo IgG release rates from gels will be similar and consistent.

**Materials and methods:** In vitro release of IRDye800CW-labeled IgG in PBS from thermosensitive pentablock co-polymers (PBC) was performed. IgG was quantitated using a microplate reader and standard curves. PBC variants included hydrophilic, hydrophobic, nanoparticles, and a PBC control. Additionally, mice were injected subcutaneously with the same PBC groups and imaged using the Xenogen IVIS system. Regions of interest were measured each day and radiant efficiencies compared.

**Results:** In vitro release revealed 50% of IgG was released by Day 2 and 100% released by 14 days. In vivo, 50% percent of the IgG was released by Day 3, with the remaining IgG released over 3 weeks. Hydrophobic PBC resulted in IgG fluorescence for more than 3 weeks. Results from IgG nanoparticles in PBC are pending.

**Conclusions:** In vivo imaging allowed real-time evaluation of IgG release and comparison of release rates from different gel formulations. In vivo IgG release from PBC was slower and more controlled compared to in vitro release. Hydrophobic PBC had more sustained release of IgG in vitro and in vivo.

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INVESTIGATION OF ENTEROPATHOGENIC E. COLI INFECTION IN KITTENS WITH DIARRHEA-ASSOCIATED MORTALITY

Victoria E. Watson, graduate student
Megan E. Jacob, Chitrita DebRoy, James R. Flowers, James S. Guy, Stephen H. Stauffer, and Jody L. Gookin
vewatson@ncsu.edu
NCSU CVM

Infectious diarrhea is a leading cause of death in children and kittens. In children, enteropathogenic E. coli (EPEC) is a common cause of diarrhea and is especially associated with risk of mortality. We identified adherent EPEC in intestines of sick kittens that died in animal shelters. We hypothesize that EPEC is common in shelter kittens and is significantly associated with diarrhea-related mortality.

We performed a prospective, case-controlled study investigating naturally-occurring EPEC infection in kittens that died with diarrhea and control kittens without diarrhea. E.coli was cultured from colonic contents and pathotyped by PCR. EPEC were screened for bfp, serotyped, pulsotyped, and evaluated for motility. PCR was performed on fecal DNA for amplification of eae (E.coli attaching and effacing). Histopathology of the gastrointestinal tract was scored for inflammation and assessed for adherence of bacteria.

Atypical EPEC (eae-positive, bfp/stx-negative) were isolated from 6/26 (23%) kittens with diarrhea-associated mortality and 2/16 (12.5%) control kittens. Diverse serotypes and pulsotypes of EPEC were identified. Eae was PCR-amplified from feces in 18/31 (58%) kittens with diarrhea and 3/17 kittens (18%) without diarrhea (p<0.01;X^2). Quantitative PCR demonstrated a greater quantity of eae in feces from kittens with diarrhea. Scores of small intestinal inflammation were significantly higher in kittens dying with diarrhea (p<0.01;t-test).

This study identified EPEC as common in kittens and demonstrates a significant association between presence of eae in feces and diarrheal death. Future studies investigating the causal relationship between EPEC and diarrhea-associated mortality in shelter kittens may help ameliorate the death toll of diarrhea in kittens.

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The immunoglobulin (Ig) superfamily (IgSF) includes membrane bound and secreted proteins that possess one or more Ig domains that share core conserved residues and structural features with immunoglobulins (e.g. antibodies). Numerous IgSF members play significant roles in immune function. Hundreds of IgSF members have been identified within genomes of various teleost fish species. For example, novel Ig-like transcripts (NILTs) that encode membrane bound proteins possessing one or two Ig domains have been identified in carp, salmon, and trout. Although NILTs can encode either activating or inhibitory signaling motifs, their cellular function remains unknown. Three predicted genes encoding NILTs were previously described on chromosome 1 of the model species, zebrafish (Danio rerio). Data mining the surrounding genomic sequence of the predicted NILT genes revealed a cluster of >100 uncharacterized immunoglobulin domains phylogenetically related to NILTs that spanned ~1.2 Mbp of DNA. We have sequenced transcripts corresponding to 21 of these genes and are actively cloning additional transcripts. Our sequence data suggests polymorphic and haplotypic variation for these genes. We are pursuing the targeted genomic disruption of select members of this gene cluster. Once stable zebrafish lines are generated, we can assess the relevance of these genes to immune function with in vivo assays. Ascertaining what role these genes play in zebrafish immunity will shed important light onto their role in all teleost fish, including aquaculture species, and is a direct aim of our research.
NONTARGETED METABOLOMIC INVESTIGATION OF CAPTIVE HARD (ACROPORA SP.) AND SOFT (LOBOPHYTUM SP.) CORAL IN GOOD AND COMPROMISED WATER CONDITIONS USING $^1$H-NMR SPECTROSCOPY

Lori S. H. Westmoreland$^{1,3}$, House Officer

Jennifer N. Niemuth$^{1,3,4}$, Hanna Gracz$^{1,2}$, Michael K. Stoskopf$^{1,3,4}$

lswestmo@ncsu.edu, jennifer_niemuth@ncsu.edu, hanka.gracz@gmail.com, mkstosko@ncsu.edu

Department of Clinical Sciences, College of Veterinary Medicine, NCSU
Department of Biochemistry, College of Sciences, NCSU
Environmental Medicine Consortium, NCSU
FWCB Graduate Program, NCSU

Global coral reef decline attributed to anthropogenic, climate, and environmental impacts has stimulated interest in coral health. We used proton nuclear magnetic resonance spectroscopy ($^1$H-NMR)-based metabolomics to investigate potential markers of health of a captive hard (Acropora sp.) and soft coral (Lobophytum sp.) under compromised water conditions. We hypothesized hard and soft coral metabolomes would reflect physiologic differences based on their distinct anatomy, and that pan-tissue metabolomic analysis would be sufficiently sensitive to evaluate coral metabolic responses to deteriorated water parameters. Small samples collected from the distal tips of hard and soft coral colonies acclimated to good and then exposed to deteriorated water quality conditions (increased ammonia and phosphate, decreased calcium) were flash frozen. Metabolites were extracted using a newly developed, environmentally sound, amphibian Ringer’s solution 2:1 (volume:weight) method. 1-D and 2-D NMR spectra were obtained using micro and macro coil techniques on experimental Bruker magnets. Metabolites identified in soft and hard coral including osmolytes, amino acids and lipids were similar, supporting a broadly conserved metabolome. Under compromised water conditions tissue concentrations of lactate and thymine decreased, while trigonelline, acetate, and acrylic acid increased. Interpretable data obtained from very small coral samples using $^1$H-NMR spectroscopy demonstrated that coral metabolism is impacted by subtle environmental stressors such as poor water quality. Studies establishing metabolomic profiles for key coral species will be critical to evaluate physiologic status and health of coral reefs experiencing environmental change, disease outbreaks and other natural and anthropogenic stressors.

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Handling and restraining rabbits for routine procedures may be impossible without prior sedation, or may result in unnecessary stress or injury to the rabbit or handler. Parenteral administration of sedative agents also can cause stress, as well as localized pain and tissue damage, especially in fractious patients. This study investigated the efficacy and safety of oral transmucosal (OTM) detomidine gel as an alternative to injectable sedation in rabbits. Eight adult male New Zealand White rabbits received a dose of 0.6, 1.2, or 1.8 mg/kg OTM detomidine. Physiological parameters and sedation scores (SS) were assessed at 10-minute intervals from baseline to 90 minutes, and histopathology of cardiac tissue was scored 1-12 days after dosing. SS increased in all rabbits, but only 3/8 rabbits reached effective sedation (SS of 10 based on 5 reflex responses on a 0 to 3 scale). SS did not differ among dosage groups and the time/dose interaction was not statistically significant. Heart rate decreased rapidly in all rabbits, with no difference among dosage groups, and there was no effect of time or dosage on SpO2. Minimal to mild degenerative changes were seen in the myocardium of treated rabbits, but there was no myocyte necrosis, inflammation, fibrosis, or mural thrombi, as reported previously in rabbits that received parenteral detomidine. OTM detomidine gel was safely and easily administered, but duration and level of sedation were unpredictable. Therefore, its use as a sole option to facilitate handling and restraint of rabbits does not offer advantages over existing parenteral regimens.
EVALUATING MILK CELLULAR RESPONSE TO INTRAMAMMARY AND TOPICAL HERBAL PRODUCTS.

V. L. Wolf¹: Veterinary Student, vlwolf@ncsu.edu
S. P. Washburn², R. Baynes¹, K. Mullen², M. Correa¹ and K. L. Anderson¹

¹North Carolina State University College of Veterinary Medicine, Raleigh, NC
²North Carolina State University College of Agriculture and Life Sciences, Raleigh, NC

With the growth in the sale of organic milk products, alternative mastitis and dry cow therapies are needed in the organic dairy industry to maintain the udder health of cattle. Phytoceuticals and other plant-derived treatments are often used instead of antibiotics. Few, if any, studies have characterized the response to herbal products used in cattle. Two plant-derived products were studied in 17 lactating organic cows as part of a larger on-going study of herbal product residue kinetics. Cows were treated with either an intramammary (IMM) product (7 cows; Phyto-Mast®, a botanical formulation for udder health, CowMaster, LLC., PA) or a topical (TOP) product (10 cows; Uddersol™, a blend of essential oils, Ralco Animal Nutrition, Marshall, MN). Baseline milk data were collected, treatments were administered to randomly selected quarters with the contralateral quarter serving as untreated control, and milk cellular response was followed over time post-treatment. Quarter milk values for total leukocyte count, neutrophils, lymphocytes, and macrophages were obtained. Data were analyzed at selected time periods after treatment and mean and standard deviations determined. Preliminary inspection of the leukocyte data indicates that IMM-treated quarters demonstrated some degree of milk cellular response compared to TOP herbal product. Repeated measures analysis of variance was performed resulting in a marginal p-value of 0.06 for the treatment by time interaction term for total leukocyte count. The project and data analysis are on-going. The results suggest that IMM administration of herbal products may stimulate a degree of cellular reaction in the bovine mammary gland.

Funded by NCSU CVM Merial Veterinary Scholars Program, NCSU CVM Office of the Associate Dean of Research, and National Institute of Food and Agriculture in the U.S. Department of Agriculture.
Many internal organs are left-right asymmetric in their configuration within the vertebrate body, including the liver, whose left and right lobes exhibit differences in both size and anatomical morphology across species. As many as 1 in 10,000 humans are born with defects in left-right asymmetry that often involve severe anomalies in liver laterality, yet the mechanisms underlying the development of left-right asymmetries in the liver are virtually unknown. We sought to determine how liver asymmetries are established during hepatogenesis in the model frog species *Xenopus laevis*. *Pitx2c*, a homeobox transcription factor, is expressed on the left side of the developing heart, lungs and gastrointestinal tract, and is required for proper asymmetrical morphology in these systems. We hypothesized that *Pitx2c* is required for asymmetric liver development. We found that *Pitx2c* is expressed in the left mesoderm surrounding the left hepatic diverticulum and that experimentally induced, right-sided expression of *Pitx2c* perturbed liver asymmetry. We also discovered that the liver specific transcription factor, *Hhex*, has a broader expression domain on the right side of the animal than the left and that experimentally induced, right-sided *Pitx2c* disrupted this expression. These results show that asymmetrical *Pitx2c* expression is required for hepatobiliary asymmetry and suggests that *Pitx2c* orchestrates this asymmetry by limiting the expression of organ specification genes like *Hhex*. Our results advance our understanding of how asymmetries develop in all left-right asymmetric organs and give us insight into birth defects involving laterality.

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