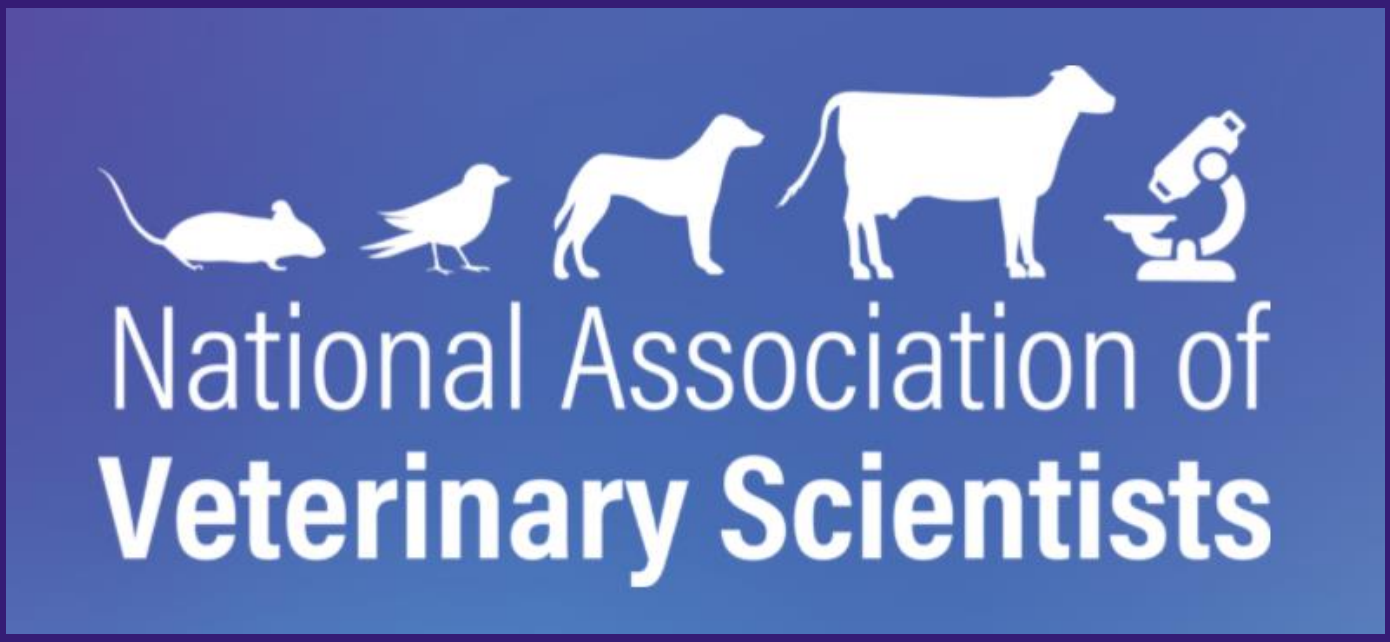


6th Annual Combined DVM/PhD Degree Colloquium

Interdisciplinary Research & Collaboration

August 4th, 2022; St. Paul, MN

Program Directory

NATIONAL ASSOCIATION OF VETERINARY SCIENTISTS

6th Annual Combined DVM/PhD Degree Colloquium

Interdisciplinary Research and Collaboration

August 4th, 2022, St. Paul, MN

National Association of Veterinary Scientists

Featured Speakers

Keynote: Dr. Chand Khanna
Chief Science Officer, Ethos Veterinary Health
President, Ethos Discovery

Keynote: Dr. Johanna Salzer
Epidemiology Team Lead
Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, CDC

Workshop: Dr. April Kedrowicz
Assoc. Prof. of Clinical Communications
NC State

Panelist: Dr. Erin Tsi-Jia Chu
Life Sciences Lead for Open Data
Amazon Web Services

Panelist: Dr. Brian Leonard
Assist. Prof. Veterinary Ophthalmology
UC Davis

Schedule

August 3rd, 2022

6:00-8:00pm Welcome Reception in Atrium at DoubleTree
411 Minnesota Street / St. Paul, MN 55101

August 4th, 2022

Conference Venue: St. Paul RiverCenter
175 West Kellogg Boulevard / St. Paul, MN 55102

8:00-8:30am	Registration and Refreshments
8:30-8:45am	Welcome Address
9:00-10:00am	Keynote Addresses
10:00-10:50am	Poster Session 1
11:00-12:00pm	Student Oral Presentations Session 1
12:00-1:00pm	Lunch (Provided) & Director Meeting
1:00-2:00pm	Science Communication Workshop
2:00-2:50pm	Poster Session 2
3:00-4:00pm	Student Oral Presentations Session 2
4:00-5:00pm	DVM/PhD Career Panel
5:00-5:15pm	Closing Remarks
5:30-6:30pm	Dinner (Provided)
6:30-8:00pm	Student Social Hour

*Masks, social distancing, and vaccination are encouraged. We will follow all state/local/federal guidelines and any guidelines instituted by the venue itself based on the public health situation closer to the time of the event.

Keynote Biographies

Dr. Chand Khanna, DVM, PhD, DACVIM (Onc), DACVP (Hon)

Chief Science Officer, Ethos Veterinary Health; President, Ethos Discovery

Dr. Khanna is a veterinary oncologist, Chief Science Officer of Ethos Veterinary Health & President of Ethos Discovery, a non-profit incubator of scientific innovation. Dr. Khanna's career has included clinical practice as a veterinary oncologist, and as a scientist in the field of metastasis biology and therapy where he directed a team within the Pediatric Oncology Branch of the NCI and was the founding Director of the Center for Cancer research Comparative Oncology Program at the NIH.

Dr. Johanna Salzer, DVM, PhD

Epidemiology Team Lead, Rickettsial Zoonoses Branch, Vector-Borne Diseases, CDC

Dr. Johanna Salzer is a Veterinary Medical Officer and Epidemiology Team Lead for the Rickettsial Zoonoses Branch in the Division of Vector-Borne Diseases at CDC. She currently leads a team of epidemiologists, medical officers, and veterinarians focused on surveillance, outbreak response, prevention, and research of rickettsial diseases. Her work includes both domestic and global public health activities focused on zoonotic diseases such as Rocky Mountain spotted fever, ehrlichiosis, anaplasmosis, and Q fever. Dr. Salzer started her career at CDC in 2008 working on the Ecology Team in the Poxvirus and Rabies Branch leading both field investigations and laboratory studies in the BSL3 and BSL4. After completing her PhD at Emory University in 2014, she served as an Epidemiologist in the Bacterial Special Pathogens Branch, where she focused on supporting CDC's Global Health Security Agenda activities related to melioidosis, anthrax, brucellosis, and leptospirosis. Dr. Salzer has worked on a wide variety of zoonotic diseases and One Health issues throughout her public health career which have included partnerships in India, Cameroon, Bangladesh, Sierra Leone, Uganda, and Mexico. Dr. Salzer holds a Doctorate in Veterinary Medicine from the University of Illinois Urbana- Champaign and a PhD in Population Biology, Ecology, and Evolution from Emory University.

Workshop Coordinator Biography

April Kedrowicz, PhD

Associate Professor of Communication, Department of Clinical Sciences, NC State University

Dr. April A. Kedrowicz, (Ph.D., University of Utah) is an Associate Professor of Communication at North Carolina State University, College of Veterinary Medicine. Dr. Kedrowicz has over 20 years of experience teaching interpersonal and professional communication. She developed and coordinates NC State's clinical and professional communication curriculum. Prior to joining NCSU's College of Veterinary Medicine, Dr. Kedrowicz was the founding director of the University of Utah's CLEAR Program, an interdisciplinary engineering communication initiative. Under her leadership, an innovative communication curriculum was implemented in all accredited departments in the College of Engineering, resulting in measurable improvements in students 'communication skills. Research interests include communication education, health communication, clinician-client communication, and socialization and professional identity.

Alumni Panelist Biographies

Brian Leonard, DVM, PhD, DACVO

Assistant Professor of Comparative Veterinary Ophthalmology, U.C. Davis

Dr. Brian Leonard is originally from Rochester, NY and attended Kenyon College in Gambier, OH for his undergraduate education. He subsequently completed his DVM/PhD through a combined Veterinary Scientist Training Program at the University of California, Davis. After graduation, he completed a one-year small animal rotating internship at the University of Wisconsin-Madison, followed by a four-year residency in Comparative Veterinary Ophthalmology at the University of California and achieved board-certification. Dr. Leonard is an Assistant Professor at the University of California, Davis, a Co-Principal Investigator in the Murphy-Russell Leonard-Thomasy Laboratory, serving as a clinician scientist with appointments in the MRLT research lab and the Comparative Ophthalmology Service of the Veterinary Medical Teaching Hospital. His research is focused on ocular surface disease, with emphasis on microbial keratitis, corneal wound healing and dry eye disease. Keratorefractive surgery is commonly performed worldwide as a means for improving vision, however the outcome is dependent on a highly coordinated wound healing response. During healing, corneal keratocytes transdifferentiate into myofibroblasts, and aberrant persistence of the myofibroblast phenotype is associated with corneal fibrosis and poor patient visual outcomes. Dr. Leonard's research is centered on uncovering the signaling pathways that drive the keratocytes to myofibroblast transdifferentiation and exploring complex interaction between these cells and the extracellular matrix of the corneal stroma. The goal of his research is to modulate these signaling pathways to orchestrate the wound healing process, prevent the development of corneal fibrosis and ultimately, improve patient outcomes.

Erin Tsi-Jia Chu, DVM, PhD

Life Sciences Lead for Open Data, Amazon Web Services

Erin Chu (Cornell DVM '14, PhD '17) is the Life Sciences Lead for Open Data at Amazon Web Services (AWS). Erin tracked equine in vet school and completed her PhD studying DNA methylation in the mouse male germline. Concurrently, she consulted for, and later joined full time, with Embark Veterinary, a dog genomics startup where she did everything from experimental design to product development to customer support. At AWS, Erin works with leading researchers in academia, nonprofit, and industry to speed their time to science by shaping data strategy and devising cloud-native solutions. Erin is based in Austin, TX with her husband Evan and her Belgian Tervuren Ursa.

Student Oral Presentations

Session 1 (11:00am-12:00pm)

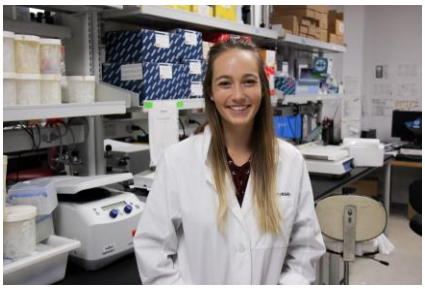
Kieran Koch-Laskowski

Cornell University

Kieran grew up in the Greater Philadelphia area and completed her undergraduate studies at the University of Pennsylvania. After graduating, she continued to cultivate her interests in biomedical research and veterinary medicine as both a lab technician at Penn as well as an assistant at a local small animal hospital. She was grateful for the opportunity to pursue both fields as part of Cornell's Combined DVM/PhD program. Now in her fifth year of the program, Kieran studies how dietary, cellular, and molecular factors interact in the gut and influence overall metabolic health. Her research focuses on uncovering how microRNA regulation within the intestine varies under different dietary conditions and how such mechanisms may be leveraged to combat metabolic diseases. In her free time, she enjoys being active among Ithaca's great outdoors, exploring new places and cuisines, reading memoirs and fiction, and spending time with her friends, family, husband, and Nicaraguan street dog.

Charlotte Nyblade

Virginia-Maryland College of Veterinary Medicine



Charlotte Nyblade is a PhD student in the Virginia-Maryland College of Veterinary Medicine dual degree program. She attended Brown University, graduating in 2021, with a B.A. in Health and Human Biology. Her current research involves novel therapeutics and vaccines for enteric pathogens. Upon completion of the PhD component, she will enter the DVM portion of the degree where she hopes to continue focusing on infectious disease and diagnostics.

Kristina Ceres

Cornell University

Kristina is a combined DVM-PhD student at Cornell University. She recently completed her PhD in molecular epidemiology and pathogen evolution in the Gröhn lab. Her PhD research focused on using machine learning, pathogen genomics, and forward genetic simulation to study mycobacterial pathogen dynamics and evolution. During her PhD she was also involved in the evolutionary and epidemiologic analysis of other zoonotic pathogens, and most recently has been involved in shark conservation genomics work. She is currently a One Health summer fellow at Conservation X Labs working in molecular diagnostics. Kristina is interested in One Health/Planetary Health research approaches and plans to pursue opportunities in translational science related to wildlife conservation and global health after graduation.

Session 2 (3:00pm-4:00pm)

Kimberly Demos-Davies, DVM

University of Minnesota

Kimberly Demos-Davies is a PhD candidate and NIH T32 trainee at the University of Minnesota College of Veterinary Medicine. Her research focuses on investigating the distant inflammatory and neurocognitive effects of non-brain directed radiation therapy. She holds a BA in biology from the University of Colorado and a DVM from the University of Minnesota College of Veterinary Medicine.

Ankita Gupta

North Carolina State University

Ankita Gupta is a sixth-year DVM/Ph.D. student in the Comparative Biomedical Sciences graduate program at North Carolina State University, College of Veterinary Medicine. Her primary research interest is to improve the success of translational research for chronic pain. Her thesis work focuses on using validated outcome measures and molecular genetic tools in rodent models and dogs with naturally occurring osteoarthritis pain to evaluate putative analgesic targets.

Alexandra Kaloss

Virginia-Maryland College of Veterinary Medicine

Allie Kaloss is a current fourth year dual degree candidate at the Virginia-Maryland College of Veterinary Medicine. She is in the lab of Dr. Michelle Theus, where she is interested in translational neuroscience research. Her thesis work focuses on how specific receptor tyrosine kinases control vascular growth and remodeling following ischemic strokes.

Gut epithelial adaptations to dietary, genetic, and surgical perturbation dissected at single cell resolution

Kieran Koch-Laskowski, Ki-Suk Kim, Matt Kanke, Michael Shanahan, Yu-Han Hung, Rebecca Cubitt, Darleen Sandoval, and Praveen Sethupathy

Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY (Koch-Laskowski, Kanke, Shanahan, Hung, Cubitt, Sethupathy); Department of Pediatrics, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO (Kim, Sandoval)

As a critical site for nutrient absorption, barrier defense, and hormone secretion, the gut is central to the regulation of metabolic health. These diverse functions are coordinated by specialized absorptive and secretory cell lineages of the intestinal epithelium, which continuously differentiate from crypt-based stem cells. Under different metabolic contexts, epithelial function can be modified in a maladaptive or therapeutic manner, specifically by dietary intervention, genetic perturbation, or bariatric surgery. However, the mechanisms underlying these changes remain poorly characterized. To bridge this knowledge gap, we performed single-cell RNA-seq analysis of two murine models. First, our lab recently identified miR-375 as one of the most highly expressed microRNAs (miRNAs) in stem and secretory cells of the murine intestinal epithelium. Given the established and conserved role of miRNAs in regulating intestinal homeostasis, we profiled epithelial crypts and villi isolated from wildtype and miR-375 knockout mice fed either a low- or high-fat diet. We found that the loss of miR-375 exerts diet-specific effects on epithelial lineage composition and gene expression, which correspond with *in vivo* metabolic phenotypes. Second, we studied crypts and villi from a mouse model of metabolic disease subject to bariatric surgery. Preliminary results indicate that surgery rescues high-fat diet-induced imbalances in lineage allocation, especially for secretory cell types. Altogether, our findings represent the first high-resolution study of gut epithelial adaptations to genetic perturbation or bariatric surgery, which further our mechanistic understanding of metabolic disease in pursuit of more effective therapeutic options.

Research Grants: ADA 1-16-ACE-47 (awarded to PS), DK 121995 and ADA 1-19-IBS-252 (awarded to DS), 1K01DK129367-01 (awarded to KSK)
Student Support: 1F30OD031914-01 (awarded to KKL)

Gnotobiotic pig models of *Clostridioides difficile* infection and disease

Charlotte Nyblade, Viviana Parreño, Peng Zhou, Casey Hensley, Vanessa Oakes, Hassan M. Mahsoub, Kelsey Kiley, Maggie Frazier, Annie Frazier, Yongrong Zhang, Hanping Feng, Lijuan Yuan

Biomedical Sciences and Pathobiology (Nyblade, Parreño, Zhou, Hensley, Oakes, Mahsoub, Kiley, Frazier, Frazier, Yuan), Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA; Microbial Pathogenesis (Zhang, Feng), University of Maryland at Baltimore, Baltimore, MD

Clostridioides difficile (CD) is a gram-positive, spore-forming, anaerobic bacterium that is a significant One Health problem. In humans, CD is the most common cause of hospital-acquired and antibiotic-associated diarrhea. In food animals, CD causes severe enteritis, most notably in neonatal pigs. There are no vaccines available for CD. The standard antibiotic treatments often fail to prevent CD recurrence due to the persistence of spores and the emergence of antibiotic resistant strains. To develop effective preventative and therapeutic strategies, animal models that accurately represent the progression of CD infection (CDI) are necessary. Gnotobiotic (Gn) pigs are attractive models as their digestive and immune systems are physiologically similar to humans, they have a microbiota free gut, and are easily susceptible to infection. Here, we successfully established a Gn pig model of CDI and disease using the hypervirulent strain of CD UK1. UK1 infected pigs developed classic signs of CDI including severe, watery diarrhea and weight loss. CD spores and toxins were detected in feces of infected animals via anaerobic culture and cytotoxicity assays. Significant intestinal lesions were visible in the tissues of CD infected animals during in situ and histological evaluation. CDI caused upregulation of various pro-inflammatory cytokines in serum, large intestinal contents, and pleural effusion samples. This model is ready for evaluating potential preventive and therapeutic treatments, including vaccines and passive immune strategies, and will be key for addressing the global burden of CD in the healthcare and food animal industries.

Research Grant: National Institute of Allergy and Infectious Diseases, National Institutes of Health
Student Support: Institute for Critical Technology and Applied Science (ICTAS)

The evolution of antimicrobial resistance in the *Mycobacteirum tuberculosis* complex in the absence of antimicrobial use

Kristina M. Ceres, Michael J. Stanhope & Yrjö T. Gröhn

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University (Ceres, Gröhn); Population and Ecosystem Health (Stanhope), College of Veterinary Medicine, Cornell University

Antimicrobial resistant *Mycobacterium tuberculosis* is a growing threat to global health. Antimicrobial treatment selects for resistant mutations to reach high population frequencies; however, despite fitness costs associated with protein structure altering drug resistance mutations, resistance has also been found in the *Mycobacterium bovis* in animal hosts in the absence of tuberculosis-specific drug treatment. We hypothesized that both small effective population sizes during transmission events and the coevolution of neutral or low-cost structural enhancing compensatory mutations can lead to resistance evolution in the absence of positive selection by antimicrobial treatment. Using a global sample of over 3000 *M. bovis* whole genome sequences, we find that genotypic resistance is rare, but resistance to first-line tuberculosis drugs occurs in sequences from both human and animal hosts. Using forward genetic simulation incorporating predicted structural effects of specific resistance-causing mutations, we show that resistance mutations can evolve through mutation and drift and can become fixed through population bottlenecks. Our work shows that the epistatic interaction of structural stabilizing mutations and resistance-causing destabilizing mutations, and the demographic effect of population bottlenecks on allele frequency distributions can describe a mechanism for evolution of antimicrobial resistance without antimicrobial use. Resistance evolution in *M. bovis* in the absence of antimicrobial use is both theoretically and empirically rare but is possible, and the mechanism described here may demonstrate the fundamental processes governing resistance evolution before selection in the *Mycobacterium tuberculosis* complex.

Research Grant: USDA NIFA predoctoral fellowship
Student Support: USDA NIFA predoctoral fellowship

Out-of-field toxic effects of radiation therapy

Kimberly Demos-Davies, Jessica Lawrence, Davis Seelig

Department of Veterinary Clinical Sciences, University of Minnesota College of Veterinary Medicine, Saint Paul, MN (Demos-Davies, Lawrence, Seelig), Masonic Cancer Center, University of Minnesota, Minneapolis, MN (Lawrence, Seelig)

Treatment-related toxic effects are commonly reported in cancer patients undergoing radiation therapy (RT). Radiation-induced toxicity typically affects tissue within and near the irradiated field. However, tissue changes are increasingly recognized in tissues that are distant from the irradiation field, such as the development of neurological signs despite undergoing hindlimb RT alone. Precise mechanisms for distant, out-of-field, radiation toxicity are unknown. The objective of this study was to investigate the underlying mechanisms by which localized RT induces out-of-field effects. Nine-to-thirteen week old SKH1 mice were treated with a single dose of 20Gy or 30Gy radiation to the right hindlimb. Mice were euthanized at [6 hours (h), 24h, 5 days (d), 12d, 25d] post treatment. Plasma and irradiated tissues (skin, muscle, femur) were collected, along with left femur, brain, and spleen. All tissue except brain were processed for cytokine and immune cell analysis by flow cytometry and immunohistochemistry. Within brain, glial cell activation and neurogenesis were assessed by immunohistochemistry. Hypocellularity and increased reticular fibers in the irradiated bone marrow was noted 5d post treatment compared to unirradiated mice. Distant effects including decreased splenic weight and lymphocyte populations and widespread microgliosis and astrocytosis in the brain. This study is the first to evaluate temporal toxic effects in unirradiated tissues following local RT in mice. It provides evidence of clinically relevant distant tissue effects following focal RT and supports further work is needed to identify potential strategies to minimize the side effects in cancer patients.

Research Grant: None
Student Support: NIH T32 Comparative Medicine and Pathology Grant (T32OD010993)

Artemin signaling is involved in OA pain progression

Ankita Gupta, Uma Nair, Connor Thonen-Fleck, Santosh K. Mishra, B. Duncan X. Lascelles.

Translational Research in Pain Program (Gupta, Nair, Thonen-Fleck, Lascelles), Department of Clinical Sciences (Gupta, Nair, Thonen-Fleck, Lascelles), Department of Molecular Biomedical Sciences (Mishra), Comparative Pain Research and Education Centre (Mishra, Lascelles), North Carolina State University, Raleigh, NC.

Osteoarthritis (OA) is a leading cause of disability, with ~100 million US adults suffering from chronic joint pain, widespread sensitization, and decreased mobility. Clinically efficacious and safe therapeutics for OA pain are limited due to a lack of understanding of clinically relevant neural mechanisms of chronic OA pain. We have linked synovial fluid concentrations of a neurotrophic factor, artemin, to naturally occurring joint pain in dogs. Further, expression of GDNF family receptor alpha 3 (GFRα3, artemin's receptor) was increased in dog OA sensory neurons compared to controls. Despite our compelling data, no studies have elucidated the role of artemin/GFRα3 signaling in the development and maintenance of OA pain. This study explores the functional role of artemin/GFRα3 signaling in OA pain. We used the monoiodoacetate (MIA)- induced model of stifle OA pain to evaluate sensitivity to mechanical, hot, and cold stimuli and limb use at early inflammatory (day 7) and late OA (day 42) time points. At both time points, we assessed MIA-induced hypersensitivity and limb disuse at 2-, 5-, and 24-hrs. post-anti-artemin monoclonal antibody or isotype control administration. MIA-injected mice developed hypersensitivity to mechanical and thermal stimuli and had decreased limb use. Artemin sequestration reversed MIA-induced hypersensitivity and limb disuse at early inflammatory and late OA pain time points. This is the first evidence demonstrating the functional role of artemin/GFRα3 signaling in MIA-induced OA pain progression. Our ongoing work elucidates putative targets for developing novel, safe, and clinically effective analgesics for OA pain.

Research Grant: salary release for Lascelles
Student support: Donations to the Translational Research in Pain Program

A tale of two proteins: How EphA4/Tie2 crosstalk influences blood vessel growth following ischemic stroke

Alexandra M. Kaloss, Kennedie Lyles, and Michelle H. Theus

Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA (Kaloss, Theus); School of Neuroscience (Lyles), Virginia Tech, Blacksburg, VA.

Strokes are a leading cause of death and disability worldwide and are most commonly ischemic in nature, in which blood flow is stopped. Following blockage of the vessel, often by a blood clot, cells in the affect region rapidly die, resulting in neurological impairment. Current treatments are limited to removing or dissolving the clot, but do not necessarily restore blood flow and prevent cell death; therefore, novel treatments are needed to specifically target restoration of blood flow. Specialized, pre-existing blood vessels called pial collaterals can ease the loss of blood flow by remodeling into larger vessels after an ischemic event. Pial collateral growth is critical in preventing cell death in the initial hours after stroke but remains poorly understood. Our previous work has shown that Tie2, a protein involved in vascular stability and growth, is inhibited following stroke by other receptors, including EphA4, thereby limiting pial collateral growth and worsening outcome. To test if artificial stimulation of Tie2 can overcome this inhibition, we utilized vasculotide, an Angiopoeitin-1 mimetic peptide that activates Tie2. *In vitro* endothelial cells treated with vasculotide displayed improved migration and wound healing. Moreover, in an *in vivo* mouse model, animals that received vasculotide directly following a surgically induced ischemic stroke had significantly larger collateral vessels and reduced tissue damaged compared to PBS treated controls. Therefore, inhibiting EphA4 or stimulating Tie2 may be options for treating stroke by helping these important vessels expand and return vital blood supply to the injured areas.

Research Grant: NINDS R01NS112541 (MHT)
Student Support: Virginia-Maryland College of Veterinary Medicine dual degree program

Student Poster Presentations
August 4th 2022

Poster Session 1

10:00-10:50 am

Poster Session 1	Poster Number	Title of Presentation
Ashley	1-1	Evaluating *Sarcocystis neurona* genotypes as drivers of mass mortality events in southern sea otters
Baker	1-2	Moving targets: how Porcine Reproductive and Respiratory Syndrome Virus epitopes shift over time
Cao	1-3	Canine osteosarcoma as a translational model for advancing solid tumor CAR T cell therapy
Ceres	oral only	The evolution of antimicrobial resistance in the *Mycobacteirum tuberculosis* complex in the absence of antimicrobial use
Cockey	1-5	Engineering chimeric antigen receptor (CAR) lymphocytes to target feline infectious peritonitis virus
Dearing	1-6	Prefrontal–medullary circuit inhibition dysregulates autonomic and endocrine responses to stress
Dombroski	1-7	Determining the impact of GIP receptor signaling on alpha cell GLP-1 production
Gagliardi	1-8	Development of a novel *in vitro* fibronectin fragment equine model of osteoarthritis for translational use
Gretler	1-9	Characterization of murine infection with mutant *Salmonella enterica* serovar Typhi: a model for Typhoid fever
Gupta	1-10	Artemin signaling is involved in OA pain progression
Haines	1-11	Osteosarcoma exosome priming of alveolar macrophages promotes formation of a pre-metastatic niche
Hisey	1-12	Investigation of a novel targeted therapeutic for the treatment of evaporative dry eye disease in a murine model
Azarkevich (Chiusano)	1-13	Predicting protection from Equine Herpesvirus-1 Myeloencephalopathy using antibody sub-isotype responses
Demos-Davies	oral only	Out-of-field toxic effects of radiation therapy

Evaluating *Sarcocystis neurona* genotypes as drivers of mass mortality events in southern sea otters

Elizabeth Ashley, Devinn Sinnott, Melissa Miller, and Karen Shapiro

UC Davis School of Veterinary Medicine, Davis, CA (Ashley, Sinnott, Shapiro); Marine Wildlife Veterinary Care and Research Center, California Department of Fish and Wildlife, Santa Cruz, CA (Miller)

Southern sea otters are a threatened keystone and sentinel species found along the California coast. *Sarcocystis neurona* is a protozoan parasite that poses a unique threat to sea otters in that it causes not only annual sporadic deaths but also periodic mass mortality events. It has been postulated that, following intense rainfall, *S. neurona* oocysts in opossum (the definitive host of *S. neurona*) feces are transported to the nearshore via runoff. When this occurs, some combination of parasite, host, and environmental factors may act synergistically to incite sea otter mortality events. To evaluate differences in parasite genotypes between epizootic and sporadic mortalities due to *S. neurona*, we will compare the genotypes of *S. neurona* associated with sea otter mortality events (n=22) to previously published *S. neurona* genotypes isolated from sporadic *S. neurona* deaths in sea otters, as well as from other marine and terrestrial host species (n=47). Identifying potential associations between *S. neurona* genotypes and the occurrence of mortality events will address unresolved questions on the role of pathogen virulence in fatal *S. neurona* epizootics. Understanding the unique molecular signature of virulent *S. neurona* genotypes could facilitate rapid detection and intervention at the onset of mortality events, as well as provide prognostic information for otters in rehabilitation.

Research Grant: Morris Animal Foundation
Student Support: NIH T35

Moving targets: how Porcine Reproductive and Respiratory Syndrome Virus epitopes shift over time

Julia P. Baker, Kim VanderWaal, Igor Paploski, Albert Rovira, Dwain Guggenbiller, and Ronald Kaptur

Comparative and Molecular Biosciences (Baker), Veterinary Population Medicine (VanderWaal, Paploski), Veterinary Diagnostic Laboratory (Rovira), University of Minnesota College of Veterinary Medicine, Saint Paul, MN; Phibro Animal Health (Guggenbiller, Kaptur), Teaneck, NJ

PRRSV is the most economically significant disease of swine in the US, with industry-wide losses estimated at $664 million annually. Despite increased effort to understand this virus, vaccination and other control methods persistently fail in preventing outbreaks. PRRSV evolves rapidly which poses a major barrier to effective vaccine development by generating genetic and antigenic diversity leading to poor cross-protection between different strains and vaccines. To date, an effective, universal vaccine remains elusive. The surface protein GP5 is of particular interest for immunity because it contains several proposed epitopes and demonstrates substantial variability in the viral population. Having a better understanding of how key GP5 regions vary across time is likely key to understanding the cycles of how different PRRSV strains emerge and cause disease in endemic herds. Using an epitope pattern scheme, 'MJPRRS' developed by Phibro Animal Health, we sought to investigate whether these epitope patterns clustered to viral lineages. We have found that older lineages and a widely used vaccine strain tend to contain epitopes classified as epitope pattern P1, whereas strains that emerged more recently tend to contain epitopes primarily classified as epitope patterns P4 and P5. Currently, we are investigating if the epitope patterns associated with PRRSV lineages are stable, whether they change over time, and if such changes from one pattern to another are predictable. This analysis of epitope patterns will allow us to develop epitope-informed models of evolution which could be used to allow for prediction of dominant epitopes and lineages.

Research Grant: NIFA-NSF-NIH Ecology and Evolution of Infectious Disease
Student Support: None

Canine osteosarcoma as a translational model for advancing solid tumor CAR T cell therapy

Jennifer W Cao, Jessica Lake, Renata Impastato, Lyndah Chow, Jade Kurihara, Dylan Ammons, Ashley Yingst, Michael Verneris, Steven Dow

Microbiology, Immunology and Pathology (Cao, Ammons, Dow), Clinical Sciences (Impastato, Chow, Kurihara) Colorado State University, Fort Collins, CO, U.S.A.,
Hematology/Oncology/BMT (Lake, Yingst, Verneris), University of Colorado Anschutz Medical Campus, Aurora, CO, U.S.A

Canine Osteosarcoma (OS) has been used as a translational model for pediatric OS due to similar presentation, molecular markers and clinical progression with high rates of metastasis to the lungs. The checkpoint molecule B7-H3 is upregulated in human and canine OS and correlates with poor prognosis, increased metastasis and decreased tumor infiltrating lymphocytes in both species. CXCL8 a chemokine that binds to CXCL2 is secreted in high amounts by a proportion of both human and canine OS which correlates to poor prognosis and greater metastatic potential. Chimeric Antigen Receptor (CAR) T cell therapy allows for the targeting of a specific surface antigens to generate an adaptive immune response. Despite being able to achieve complete remission in patients with B cell malignancies, clinical trials in solid tumors have not shown the same favorable outcomes. Immune suppression by myeloid cells within the tumor microenvironment (TME) and CAR T cell homing from the circulation to the tumor are challenges unique to solid tumors. This study aims to develop metastatic canine osteosarcoma as a translational model to evaluate enhancing the efficacy of CAR T cell therapy by immune suppressor cell depletion and increasing CAR T cell signaling by dual valent B7-H3-CXCR2 CAR. We found that dual valent CAR T cells were activated by canine B7H3 positive tumors with increased activity against high CXCL8 secreting tumors. CAR T cell activity was evaluated by secreted proinflammatory cytokines, direct tumor killing and migration to CXCL8 secreting tumor cells. Repurposed drugs losartan and propranolol in combination significantly decreased the infiltration of immune suppressive TAMs within xenograft canine OS tumors in SCID-beige mice.

Research Grant: V foundation
Student support: V foundation

Engineering chimeric antigen receptor (CAR) lymphocytes to target feline infectious peritonitis virus

James R. Cockey, Natalia Lopez-Barbosa, Gary R. Whittaker, Matthew P. DeLisa, and Cynthia A. Leifer

Department of Microbiology & Immunology, College of Veterinary Medicine (Cockey, Whittaker, and Leifer), Robert Frederick Smith School of Chemical and Biomolecular Engineering (Lopez-Barbosa and DeLisa), Cornell University, Ithaca, NY.

The fatal disease feline infectious peritonitis (FIP) currently has no FDA approved treatments. The goal of this study is to design a novel immunotherapy targeting cells infected with FIP virus (FIPV) and thus expressing surface spike protein that can be detected by chimeric antigen receptor (CAR)-engineered immune cells. CAR immunotherapy has been successful in treating some human cancers but has not yet been developed for acute viral infections like FIPV, nor used at all in cats to date. CARs are comprised of two main components: a single chain antibody fragment (ScFv) and signaling domain(s) from immune costimulatory receptor(s). Here we will design an ScFv specific for FIPV spike protein to create an anti-spike CAR to direct effector immune cells to seek and destroy FIPV spike-expressing cells. Current human CAR therapies require use of autologous T cells since allogeneic T cells may attack the new host tissue and result in severe graft-versus-host disease. Thus a second goal of the study is to determine the potential to use natural killer (NK) cells which induce much less graft-versus-host disease, and thus may be used allogeneically. We have successfully demonstrated that feline T and NK cell populations can be visualized by flow cytometry and thus enriched by cell sorting. We have also designed an ScFv from the anti-spike clone 18A7.4 that is stably expressed in mammalian cells. Completion of this study will provide proof-of-principle data using an FIPV model to support the development of FIPV CAR-cell therapy for this devastating disease in cats, and will also determine the feasibility of developing a CAR-based immunotherapy for the potential treatment of acute coronaviral infections in cats and humans.

Research Grant: Cornell Feline Health Center
Student Support: Liz Hanson Graduate Scholarship

Prefrontal–medullary circuit inhibition dysregulates autonomic and endocrine responses to stress

Carley Dearing, Carlie McCartney, Ema Lukinic, Brent Myers

Department of Biomedical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

Chronic stress increases risk for metabolic disorders, such as diabetes. However, the neuroendocrine basis of how chronic stress impacts the regulation of glucose metabolism is still unknown. The purpose of this study was to determine how inhibition of the prefrontal infralimbic cortex (IL) – rostral ventrolateral medullary (RVLM) circuit influences glucose homeostasis following chronic stress. To this end, female rats with Cre-dependent expression of tetanus toxin for IL-RVLM inhibition were chronically stressed for 2 weeks or remained unstressed. These rats were then acutely challenged with a fasted glucose tolerance test (GTT). Endocrine metabolic function was evaluated during GTT by measuring blood glucose, insulin, glucagon, and corticosterone, the primary rodent glucocorticoid. Following chronic stress, circuit-intact females show insulin-dependent impaired glucoregulation characterized by decreased glucose clearance, elevated corticosterone, and insulin insensitivity. Inhibition of IL-RVLM circuit also impaired glucoregulation regardless of stress status. However, in unstressed animals with circuit inhibition, this impairment was sympathetically mediated with no compensatory insulin response and elevated glucagon. Chronically stressed females with circuit inhibition show both sympathetic dysregulation and evidence of parasympathetic insulin compensation, indicating broader autonomic dysregulation and disruption of counter-regulatory mechanisms involved in glucose homeostasis. Collectively, these data indicate chronic stress leads to insulin-dependent glucodysregulation and the IL RVLM circuit is necessary for maintaining sympathovagal balance in glucose homeostasis in female rats. Studies in males are ongoing.

Research Grant: NIH grant R01 HL150559
Student Support: NIH grant F30 OD032120

Determining the impact of GIP receptor signaling on alpha cell GLP-1 production

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Insulin secretion in response to oral glucose intake is described as the incretin effect and is driven by two gut-derived hormones: glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). GLP-1 is derived from the protein proglucagon, which can be cleaved into either glucagon or GLP-1 via the PC2 or PC1/3 enzyme, respectively. Traditionally, it was believed that PC1/3 expression was exclusive to the gut, but multiple studies have demonstrated alpha cells are capable of being stimulated to express PC1/3 and consequently produce GLP-1. Because the half-life of active GLP-1 in circulation is extremely short, it is believed alpha cells play a role in promoting glucose-stimulated insulin secretion (GSIS) via paracrine signaling to beta cells using GLP-1. A recent study demonstrated that GIP contributes to GSIS through the alpha cell. We hypothesize alpha cell GIP receptor signaling promotes GSIS by activating the production of GLP-1. The goal of our project is to mimic the conditions of this study to determine if GLP-1 is released in response to alpha cell GIP receptor signaling, which would give more insight into potential mechanism behind alpha and beta cell communication in GSIS. To achieve this, we will treat alpha TC1-6 cells with GIP under conditions of high and low glucose and measure the alpha cell response via ELISAs for glucagon, active GLP-1 and insulin levels. Additionally, PC1/3 & PC2 mRNA and protein levels will be quantified by qPCR and immunoblotting. Because glucogenic amino acids, such as alanine, are key stimulants of alpha cell hormone secretion, treatments containing GIP and alanine will be analyzed as described above as well.

Research Grant: Hartwell Foundation, NIH/NIDDK R56DK124853
Student Support: UC Davis SVM Endowment Funds, NIH Grant T32GM136559

Development of a novel *in vitro* fibronectin fragment equine model of osteoarthritis for translational use.

Rachel Gagliardi, Richard Loeser, Lauren Schnabel

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Osteoarthritis (OA) remains the most common joint disorder worldwide, but despite the major quality-of-life burdens that OA causes there are still limited therapeutic options available. A significant challenge to the development of new therapeutics is the inconsistency of traditional *in vitro* models, which invoke an intense but transient and extremely variable inflammatory response. Our goal is to establish a novel equine *in vitro* fibronectin fragment stimulated model of OA for translational use. Our collaborator, Dr. Loeser, has recently demonstrated that fibronectin fragments (FN-f), produce an OA phenotype in human chondrocytes *in vitro*. We hypothesize that FN-f stimulation will result in a reliable equine OA model by consistently inducing significant alterations to the transcriptional profile of equine synoviocytes and chondrocytes that closely resembles the phenotype of age-related OA. Equine synoviocytes and chondrocytes will be grown in monolayer and co-culture and stimulated with FN-f for 6h or 18h, as previously determined by work done in the Loeser lab. Cells stimulated with FN-f will be compared to unstimulated cells as well as cells stimulated with traditional stimulants such as LPS and IL-1b. Preliminary data has shown that FN-f stimulation of equine synoviocytes and chondrocytes increases expression of pro-inflammatory cytokines such as IL-1b and matrix metalloproteases such as MMP3 and MMP13. Further investigation is underway to characterize the transcriptional profile of equine synoviocytes and chondrocytes stimulated with FN-f.

Research Grants: F.O.R.G.E. Fund for Orthopedic Research in honor of Gus and Equine athletes, The Barton & Marie-Claude White Equine Orthopedic Research Endowment
Student Support: GAANN fellowship in molecular biotechnology, NCSU CVM Comparative Medicine Institute, NCSU CVM DVM/PhD Program

Characterization of murine infection with mutant *Salmonella enterica* serovar Typhi: a model for Typhoid fever

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Typhoid fever is a disease specific to humans caused by infection with the bacteria *Salmonella enterica* serovar Typhi. Due to the strict host-specificity of the bacteria, the mouse models available to study typhoid fever are limited to humanized mice, which are expensive and time consuming to develop, or the use of the related bacterium *Salmonella enterica* serovar Typhimurium. This study aims to characterize murine infection of a mutant *S.* Typhi strain able to overcome the human host-restriction. The mutant *S.* Typhi contains the *ripABC* gene from *S.* Typhimurium, allowing replication of the bacteria within murine macrophages. Immunosuppressed HPS4$^{-/-}$ mice will be orally inoculated with wildtype *S.* Typhi, *S.* Typhi-*ripABC*, or *S.* Typhimurium, and bacterial load in various tissues assessed. In contrast to *S.* Typhi, *S.* Typhimurium infects both humans and mice, but causes a distinct pathophysiology from *S.* Typhi and between the two species. It is expected that the addition of *ripABC* in the permissive HPS4$^{-/-}$ mice will allow replication of *S.* Typhi-*ripABC*, with *S.* Typhi as a negative control and *S.* Typhimurium as a positive control. Additional characterization of the disease pathology, bacterial localization in tissues, and systemic response to *S.* Typhi-*ripABC* will also be assessed using histopathology, immunohistochemistry, and qRT-PCR of inflammatory cytokines. Through these methods, the ability of *S.* Typhi-*ripABC* to replicate and cause disease within the mouse will be evaluated and compared to human-restricted *S.* Typhi. The implications of this study will be the potential for this mutant strain of *S.* Typhi to be utilized as an effective model of typhoid fever in the mouse.

Research Grant: Unknown
Student Support: University of California-Davis SVM Endowment Funds

Artemin signaling is involved in OA pain progression

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Translational Research in Pain Program (Gupta, Nair, Thonen-Fleck, Lascelles), Department of Clinical Sciences (Gupta, Nair, Thonen-Fleck, Lascelles), Department of Molecular Biomedical Sciences (Mishra), Comparative Pain Research and Education Centre (Mishra, Lascelles), North Carolina State University, Raleigh, NC.

Osteoarthritis (OA) is a leading cause of disability, with ~100 million US adults suffering from chronic joint pain, widespread sensitization, and decreased mobility. Clinically efficacious and safe therapeutics for OA pain are limited due to a lack of understanding of clinically relevant neural mechanisms of chronic OA pain. We have linked synovial fluid concentrations of a neurotrophic factor, artemin, to naturally occurring joint pain in dogs. Further, expression of GDNF family receptor alpha 3 (GFRα3, artemin's receptor) was increased in dog OA sensory neurons compared to controls. Despite our compelling data, no studies have elucidated the role of artemin/GFRα3 signaling in the development and maintenance of OA pain. This study explores the functional role of artemin/GFRα3 signaling in OA pain. We used the monoiodoacetate (MIA)-induced model of stifle OA pain to evaluate sensitivity to mechanical, hot, and cold stimuli and limb use at early inflammatory (day 7) and late OA (day 42) time points. At both time points, we assessed MIA-induced hypersensitivity and limb disuse at 2-, 5-, and 24-hrs. post-anti-artemin monoclonal antibody or isotype control administration. MIA-injected mice developed hypersensitivity to mechanical and thermal stimuli and had decreased limb use. Artemin sequestration reversed MIA-induced hypersensitivity and limb disuse at early inflammatory and late OA pain time points. This is the first evidence demonstrating the functional role of artemin/GFRα3 signaling in MIA-induced OA pain progression. Our ongoing work elucidates putative targets for developing novel, safe, and clinically effective analgesics for OA pain.

Research Grant: salary release for Lascelles
Student support: Donations to the Translational Research in Pain Program

Osteosarcoma exosome priming of alveolar macrophages promotes formation of a pre-metastatic niche

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Osteosarcoma (OS), the most common primary malignant tumor of bone, often progresses to a highly fatal metastatic disease of the lungs with limited treatment options. The development of effective therapies for OS lung metastasis is limited by our understanding of the basic mechanisms driving this process. It has been suggested that prior to circulating tumor cell arrival, resident cells are "primed" to support metastasis by factors secreted by the primary tumor. These factors promote a tumor-permissive microenvironment known as a "pre-metastatic niche". Among these secreted factors are nano-sized extracellular vesicles called exosomes which are known to elicit tumor-promoting changes in tissue-resident cells in several metastatic cancer types. However, the role of exosomes in modulating the lung microenvironment during OS is not well understood. Our data shows that OS exosomes display a specific tropism for the lung supporting their hypothesized role as early drivers of pre-metastatic niche formation. *We hypothesize* that resident alveolar macrophages (AMs) are a target of OS exosomes and that primed AMs subsequently orchestrate tumor-permissive immunological changes in the lungs. To investigate this, we evaluated OS exosome biodistribution in mice using intravital imaging, multi-parameter flow cytometry, and immunofluorescence. We also investigated the effects of OS exosomes on the lung microenvironment in mice and in primary human donor-derived AMs. We show that OS exosomes can be taken up by AMs and elicit distinct changes in tumor-promoting cytokines. Our findings demonstrate a novel role for AMs as drivers of pre-metastatic niche formation during OS.

Research Grant: National Institutes of Health RO3OD028265, The Boettcher Foundation, and Webb Waring Biomedical Research Award
Student Support: National Institutes of Health Medical Scientists Training Program T32GM136628-0

Investigation of a novel targeted therapeutic for the treatment of evaporative dry eye disease in a murine model

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Evaporative dry eye disease (EDED) is characterized by tear film abnormalities, most commonly in the lipids, leading to instability and increased evaporation. We have generated a murine model of EDED by knocking out a key enzyme in tear film lipid synthesis, *acyl-CoA: wax alcohol acyltransferase 2* (*Awat2*). This study determined the clinical efficacy of a novel rabbit nonpolar lipid (rNPL), derived from the rabbit Harderian gland, in the *Awat2* knockout mice. Animals were treated 1-2 times daily in both eyes with the rNPL (n=3) or vehicle (n=2) starting at 2 months of age. Clinical examinations (slit lamp biomicroscopy), disease scoring and tear film diagnostics (phenol red thread test) were performed monthly for 1 year after which animals were euthanized and eyes were processed for histopathology. At baseline, all animals exhibited marked meibomian gland (MG) dilation with white inspissated meibum and fibrillar material in the tear film and there were subtle opacities noted in the superficial cornea of all animals. At each subsequent evaluation, progressive white corneal opacities were identified in the interpalpebral fissure of all animals; however, these opacities were more pronounced and dense in the vehicle treated animals compared to rNPL treated animals. Additionally, the MG dilation in the rNPL treated animals was subjectively reduced over time, and most clinically apparent after 1 year of treatment. Histopathology identified basophilic subepithelial deposits in one vehicle treated globe indicative of mineral deposition. These results demonstrate that treatment with rNPL decreases clinical signs of EDED, possibly through integration into the MG where it liquifies the meibum, normalizing the secretions.

Research Grant: National Eye Institute (K08 EY028199) and UC Davis Ophthalmology & Vision Science Burns Grant (S-VSMPBAG)
Student Support: NIH Training Grant (NIH Grant T32GM136559)

Predicting protection from Equine Herpesvirus-1 Myeloencephalopathy using antibody sub-isotype responses

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Equine herpesvirus-1 (EHV-1) myeloencephalopathy (EHM) is a clinically important manifestation of EHV-1 infection, causing devastating consequences economically and emotionally. Unfortunately, while there are numerous vaccines available for EHV-1, there are currently none that are labeled to protect from EHM. Although EHM is relatively rare and occurs in approximately 10% of infected horses, previous studies have shown that the risk is higher in older (>18 years) horses. Recent data indicate that protection of horses from EHM is associated with increased Immunoglobulin G sub-isotype 4/7 (IgG4/7) and decreased immunoglobulin G sub-isotype 3/5 (IgG3/5) in serum prior to challenge infection. Further data show a correlation between older age and an increase in serum IgG3/5. Using pre-existing data along with data from a recent clinical study that included EHV-1 infected horses with and without clinical EHM, this project aims to clarify the validity of using serum concentrations of IgG3/5 and IgG4/7 to predict protection from EHM. Preliminary data shows that horses who developed EHM exhibited higher ratios of IgG3/5 to IgG4/7 both pre- and post-challenge. Together with other markers of cellular/humoral immunity this data elucidates our current understanding of the immune responses associated with the development of EHM. This information is critical for the development of future vaccines targeted to protect horses of all ages from EHM.

Research Grant: Unknown
Student Support: Boehringer Ingelheim and the Graduate School at Michigan State University

Poster Session 2

2:00-2:50 pm

Poster Session 2	Poster Number	Title of Presentation
Hommer	2-1	Methionine sulfoxide reductases (MSRs) and Fe-S cluster biogenesis
Williams	2-2	Myoblast exosome production, function, and miRNA cargo is altered by mechanical stimulation
Kaloss	2-3	A tale of two proteins: How EphA4/Tie2 crosstalk influences blood vessel growth following ischemic stroke
Koch-Laskowski	2-4	Gut epithelial adaptations to dietary, genetic, and surgical perturbation dissected at single cell resolution
Loehr	2-5	miR-291-293 as serum biomarkers for pluripotent embryonal carcinoma in a mouse testicular cancer model
Luker	2-6	Regulation of folate metabolism by miR-34a identifies a potential combination therapy against osteosarcoma
Maymi	2-7	Developmental regulation of CD8+ T cell exhaustion
Nyblade	2-8	Gnotobiotic pig models of *Clostridioides difficile* infection and disease
Park Lang	2-9	Novel immunological tools to investigate CD4+ T cell activation in the canine species
Pressman	2-10	Urea treatment of crop residues reduces enteric methane emissions from dairy cattle in Kenya and Ethiopia
Savran	2-11	Ivermectin-treated birdfeed confers dose-dependent toxicity to *Culex tarsalis* mosquitoes
Pires	2-12	Investigating the function of RAD51AP1 homologous recombination DNA repair
Walker	2-13	Pathogen interactions in polymicrobial *Escherichia coli* and *Enterococcus faecalis* extraintestinal infections

Methionine sulfoxide reductases (MSRs) and Fe-S cluster biogenesis

Alexandra Hommer, Carolyn Sevier

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Iron-sulfur (Fe-S) clusters are protein cofactors that facilitate essential cellular processes. A deficiency in Fe-S proteins or Fe-S cluster biogenesis is linked to several disorders, ranging from cancer to ataxias. Reactive oxygen species (ROS) are highly reactive molecules formed by O2 reduction. Methionine side chains are particularly susceptible to oxidation by ROS. Notably, several Fe-S biogenesis enzymes contain higher than average methionine content. The eukaryote, *S. cerevisiae,* has been a model for the study of Fe-S biogenesis. Previous work in yeast established that strains deleted for the enzymes that repair oxidized methionines (methionine sulfoxide reductases, MSRs) show an upregulation of genes involved in iron regulation and a decrease in Fe-S protein activity (Sideri et al. 2009 *Microbiology* 115:612). We hypothesize that the mitochondrial Fe-S biogenesis machinery is susceptible to methionine oxidation, which disrupts the early stages of Fe-S cluster biogenesis. To test this model, we aim first to establish a role for the mitochondrial MSR in Fe-S cluster formation. Yeast have two MSR enzymes: Mxr1 and Mxr2. Mxr1 is localized to the cytoplasm, while Mxr2 is dual localized to the cytoplasm and the mitochondria depending on start codon usage. A methionine-to isoleucine mutation in Mxr2 (Mxr2-M1L) disrupts mitochondrial Mxr2 production but maintains cytoplasmic activity. My goal is to establish if Fe levels and Fe-S protein activities are altered in cells lacking mitochondrial Mxr2 activity. If a disruption in Fe-S biogenesis can be linked to an inability to repair methionine oxidation, a new focus for the prevention of the damage to Fe-S proteins may emerge.

Research Grant: NIH R01 GM105958
Student support: None

Myoblast exosome production, function, and miRNA cargo is altered by mechanical stimulation

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Center for Regenerative and Personalized Medicine, Steadman Philippon Research Institute, Vail, Colorado (Mullen, Bahney)
Department of Health and Exercise Science, College of Health and Human Sciences, Colorado State University, Fort Collins, Colorado (LaRocca, Hamilton)

Exosomes offer a potential cell-free regenerative therapy which may replicate the benefits of MSC therapy while minimizing their risks and regulatory challenges. Our previous work demonstrated that mechanical strain improves the myogenic functions of exosomes derived from myoblasts. The goal of the current study was to investigate changes in exosomal miRNA cargo following mechanical stimulation of myoblasts. We hypothesized that exosomes derived from mechanically strained myoblasts would have differential expression of miRNAs with molecular functions involving myogenesis and skeletal muscle regeneration. C2C12 myoblasts were cultured under cyclical tension using a FlexCell FX-5000TT bioreactor alongside unstrained control myoblasts. Exosomes were then isolated from conditioned media and RNA was extracted. RNA sequencing and gene ontology enrichment analysis were performed to identify differential miRNAs and their associated molecular functions. 35 miRNAs were significantly downregulated in strained exosomes compared to static. The gene ontology (GO) terms associated with the mRNA targets of these downregulated miRNAs involved developmental, neural, cell signaling, transcriptional regulation, metabolic, and inflammatory processes. This study demonstrates that mechanical stimulation alters the miRNA cargo of myoblast exosomes and that these altered miRNAs may have biological functions impacting muscle adaptation to mechanical strain. Mechanically strained myoblast exosomes therefore hold potential as a therapeutic to improve myogenesis and skeletal muscle regeneration.

Research Grant: Laboratory of Comparative Musculoskeletal Oncology and Traumatology (Colorado State University), the Limb Preservation Foundation, and the Steadman Philippon Research Institute.
Student Support: NIH T32

A tale of two proteins: How EphA4/Tie2 crosstalk influences blood vessel growth following ischemic stroke

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Strokes are a leading cause of death and disability worldwide and are most commonly ischemic in nature, in which blood flow is stopped. Following blockage of the vessel, often by a blood clot, cells in the affect region rapidly die, resulting in neurological impairment. Current treatments are limited to removing or dissolving the clot, but do not necessarily restore blood flow and prevent cell death; therefore, novel treatments are needed to specifically target restoration of blood flow. Specialized, pre-existing blood vessels called pial collaterals can ease the loss of blood flow by remodeling into larger vessels after an ischemic event. Pial collateral growth is critical in preventing cell death in the initial hours after stroke but remains poorly understood. Our previous work has shown that Tie2, a protein involved in vascular stability and growth, is inhibited following stroke by other receptors, including EphA4, thereby limiting pial collateral growth and worsening outcome. To test if artificial stimulation of Tie2 can overcome this inhibition, we utilized vasculotide, an Angiopoeitin-1 mimetic peptide that activates Tie2. *In vitro* endothelial cells treated with vasculotide displayed improved migration and wound healing. Moreover, in an *in vivo* mouse model, animals that received vasculotide directly following a surgically induced ischemic stroke had significantly larger collateral vessels and reduced tissue damaged compared to PBS treated controls. Therefore, inhibiting EphA4 or stimulating Tie2 may be options for treating stroke by helping these important vessels expand and return vital blood supply to the injured areas.

Research Grant: NINDS R01NS112541 (MHT)
Student Support: Virginia-Maryland College of Veterinary Medicine dual degree program

Gut epithelial adaptations to dietary, genetic, and surgical perturbation dissected at single cell resolution

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As a critical site for nutrient absorption, barrier defense, and hormone secretion, the gut is central to the regulation of metabolic health. These diverse functions are coordinated by specialized absorptive and secretory cell lineages of the intestinal epithelium, which continuously differentiate from crypt-based stem cells. Under different metabolic contexts, epithelial function can be modified in a maladaptive or therapeutic manner, specifically by dietary intervention, genetic perturbation, or bariatric surgery. However, the mechanisms underlying these changes remain poorly characterized. To bridge this knowledge gap, we performed single-cell RNA-seq analysis of two murine models. First, our lab recently identified miR-375 as one of the most highly expressed microRNAs (miRNAs) in stem and secretory cells of the murine intestinal epithelium. Given the established and conserved role of miRNAs in regulating intestinal homeostasis, we profiled epithelial crypts and villi isolated from wildtype and miR-375 knockout mice fed either a low- or high-fat diet. We found that the loss of miR-375 exerts diet-specific effects on epithelial lineage composition and gene expression, which correspond with *in vivo* metabolic phenotypes. Second, we studied crypts and villi from a mouse model of metabolic disease subject to bariatric surgery. Preliminary results indicate that surgery rescues high-fat diet-induced imbalances in lineage allocation, especially for secretory cell types. Altogether, our findings represent the first high-resolution study of gut epithelial adaptations to genetic perturbation or bariatric surgery, which further our mechanistic understanding of metabolic disease in pursuit of more effective therapeutic options.

Research Grants: ADA 1-16-ACE-47 (awarded to PS), DK 121995 and ADA 1-19-IBS-252 (awarded to DS), 1K01DK129367-01 (awarded to KSK)
Student Support: 1F30OD031914-01 (awarded to KKL)

miR-291-293 as serum biomarkers for pluripotent embryonal carcinoma in a mouse testicular cancer model

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Testicular germ cells tumors (TGCTs) are the most common solid malignancy diagnosed in young men in the US. Current diagnostics for TGCTs include conventional serum protein markers, but these lack the sensitivity and specificity needed to serve as accurate markers of malignancy across all histologic TGCT subtypes. microRNAs (miRNAs) are small non-coding regulatory RNAs expressed by almost all cells in the body and can be used as biomarkers of many different diseases. In humans, miRNAs in the miR-371-373 cluster are highly expressed in the serum of TGCT patients and outperform existing serum protein markers in TGCT detection. We previously developed a genetically engineered mouse model featuring spontaneous, malignant mixed germ cell tumors consisting of pluripotent embryonal carcinoma (EC) and differentiated teratoma. Here, we report that miRNAs in the mouse miR-291-293 cluster, homologs of human miR-371-373, can be used as biomarkers for malignant TGCTs in mice. miR 291-293 were detectable in the serum of mice with malignant TGCTs but not in control non-tumor-bearing mice. Interestingly, treatment with the differentiation-inducing agent thioridazine extended the survival of mice with malignant TGCTs by eliminating the tumor-propagating EC cells within the tumors, and this greatly reduced serum miR-291- 293 levels, suggesting that EC cells specifically express and secrete these miRNAs. Quantification of serum miR-291-293 levels will be a useful tool to track disease progression and response to experimental therapeutics in our mouse model, and these findings also pave the way for future studies aimed at determining functional roles for miR-291-293 in tumor pathogenesis.

Research Grant: None
Student Support: NCI F30CA247458

Regulation of folate metabolism by miR-34a identifies a potential combination therapy against osteosarcoma

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Osteosarcoma (OS) is the predominant bone cancer in dogs and humans. Due to the highly metastatic nature of this disease, OS carries a poor prognosis, necessitating further research into alternate therapeutic approaches. An emerging field of research in cancer therapy is microRNA (miR) therapeutics. Preliminary work using a proteomic approach in canine OS cells exposed to exogenous human miR-34a showed reduced levels of multiple proteins involved in the folate metabolism pathway. The aim of this study is to validate this novel antifolate function of the tumor suppressive miRNA prodrug, BioRNA/miR-34a, in canine and human OS cells *in vitro*. This study will also evaluate the combination of miR-34a and methotrexate, another antifolate chemotherapy drug commonly used in human OS treatment, for synergistic antitumor activity. Treatment of OS cells with BioRNA/miR-34a is expected to downregulate several proteins involved in the folate metabolism pathway, including GGH, TS, MTHFD1, MTHFD2, and SHMTI. Through dual inhibition of the folate pathway, a critical component of one-carbon metabolism in cancer, this augmented treatment approach is expected to result in a greater inhibition of cell proliferation and an increased level of apoptosis in canine and human OS cells compared to either treatment alone. Mature miR-34a levels following prodrug exposure will be measured by quantitative real-time PCR, protein levels via western blot, and proliferation and apoptosis via bioreductive fluorometric assay and caspase activity, respectively. Data obtained from this study will provide insight into a potential alternate therapeutic approach, improving clinical outcomes for OS patients.

Research Grant: National Institutes of Health K01OD026526
Student Support: Students Training in Advanced Research (STAR) Program at UC Davis National Institutes of Health T35OD010956

Developmental regulation of CD8+ T cell exhaustion

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While advances in CD8+ T cell biology have facilitated revolutionary immunotherapies for cancer and chronic viruses, T cell exhaustion remains a barrier to patient health. CD8+ T cell exhaustion is a hypofunctional state characterized by loss in proliferation, cytokine production, and effector function of CD8+ T cells after chronic stimulation. An important and outstanding question is why some CD8+ T cells become more exhausted than others. Our lab showed that a previously-overlooked source of CD8+ T cell heterogeneity—developmental origin—determines T cell fate after acute infection. Adult T cells (derived from bone marrow stem cells) become memory precursors, while neonatal T cells (derived from fetal liver stem cells) become short-lived effectors. I thus hypothesized that developmental origin also regulates CD8+ T cell fate in chronic infection. To test this, I transferred neonatal and adult CD8+ T cells into recipient mice and compared their responses to a chronic virus (LCMV clone 13) via high-parameter flow cytometry. Neonatal cells became highly functional effectors, whereas adult cells became exhausted. Interestingly, neonatal cells' effector skew corresponded with enhanced proliferation and migration into tissues early in infection. Late in infection, neonatal cells produced more IFNγ and may better respond to PD-1 blockade than adult cells. To understand the functional consequences of these differences, we are currently comparing their transcriptomes and behavior in tumors. Collectively, our data suggest that developmental origin plays a deterministic role in CD8+ T cell fate during chronic infection. These findings are clinically relevant to chronic viruses and cancer in human and veterinary medicine alike.

Research Grant: R01 AI110613
Student Support: Cornell Center for Vertebrate Genomics Scholarship, F30 OD032097

Gnotobiotic pig models of *Clostridioides difficile* infection and disease

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Clostridioides difficile (CD) is a gram-positive, spore-forming, anaerobic bacterium that is a significant One Health problem. In humans, CD is the most common cause of hospital-acquired and antibiotic-associated diarrhea. In food animals, CD causes severe enteritis, most notably in neonatal pigs. There are no vaccines available for CD. The standard antibiotic treatments often fail to prevent CD recurrence due to the persistence of spores and the emergence of antibiotic resistant strains. To develop effective preventative and therapeutic strategies, animal models that accurately represent the progression of CD infection (CDI) are necessary. Gnotobiotic (Gn) pigs are attractive models as their digestive and immune systems are physiologically similar to humans, they have a microbiota free gut, and are easily susceptible to infection. Here, we successfully established a Gn pig model of CDI and disease using the hypervirulent strain of CD UK1. UK1 infected pigs developed classic signs of CDI including severe, watery diarrhea and weight loss. CD spores and toxins were detected in feces of infected animals via anaerobic culture and cytotoxicity assays. Significant intestinal lesions were visible in the tissues of CD infected animals during in situ and histological evaluation. CDI caused upregulation of various pro-inflammatory cytokines in serum, large intestinal contents, and pleural effusion samples. This model is ready for evaluating potential preventive and therapeutic treatments, including vaccines and passive immune strategies, and will be key for addressing the global burden of CD in the healthcare and food animal industries.

Research Grant: National Institute of Allergy and Infectious Diseases, National Institutes of Health
Student Support: Institute for Critical Technology and Applied Science (ICTAS)

Novel immunological tools to investigate $CD4^+$ T cell activation in the canine species

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Opportunities to identify T cell epitopes in the dog have been thwarted by a lack of immunological tools specific for the species. As a result, antigen-specific triggers in diseases that affect dogs like autoimmune disorders and allergies remain unknown. Our objective was to develop immunological tools such as: IFN-γ enzyme-linked immunosorbent spot (ELISpot) and activation induced marker (AIM) assays, and an MHC class II tetramer to help overcome these hurdles. We collected splenocytes from dogs that underwent a routine splenectomy. We used these splenocytes to develop: an ELISpot assay detecting canine IFN-γ production by stimulated T cells, an AIM assay to assess early activation by CD40L expression on $CD4^+CD5^+$ canine splenocytes using a cross-reactive anti-human monoclonal antibody, and finally, we produced the first-ever canine MHC II tetramer specific for a common canine DR allele. We confirmed the efficacy of these tools in the presence of positive and negative controls, *Staphylococcus aureus* enterotoxin B (SEB) and dimethyl sulfoxide (DMSO), respectively. SEB induces a robust canine IFN-γ ELISpot readout quantifiable in canine splenocytes. Similarly, we discovered that canine CD40L expression peaks at 3 hours post-stimulation with SEB. Finally, we confirmed the development of the first canine pMHCII tetramer through size discriminatory SDS-PAGE analysis and peptide exchange assays. Our novel canine-specific reagents will help identify and characterize antigen-specific T cells and their cognate antigens driving canine MHC class II-restricted disease, thus guiding research towards the advancement of antigen-specific immunotherapies.

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Urea treatment of crop residues reduces enteric methane emissions from dairy cattle in Kenya and Ethiopia

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Enteric fermentation in the gastrointestinal tract of ruminants is a leading anthropogenic source of methane (CH4), a powerful greenhouse gas. Various strategies to mitigate enteric CH4 emissions have been studied, including diet reformulation and feed additives. The relative impact of these strategies is greatest in low- and middle-income countries, where milk production efficiency is lower than in high-income countries and greenhouse gas emissions per unit product (emissions intensity, Ei) are greater. In East Africa, crop residues (plant materials that remain after the crop is harvested, such as straws) are a principal feed resource for dairy cattle but are of poor nutritional value. Treatment of crop residues with feed- or fertilizer-grade urea is an inexpensive method to improve their quality, but the effect of urea treatment on greenhouse gases, particularly enteric CH4 emissions, is unknown. In this study, we used an empirical modelling approach to estimate enteric and manure CH4 emissions as well as nitrous oxide emissions under amended diets in which crop residues in traditional Kenyan and Ethiopian cattle diets were treated with urea. Total annual emissions under amended diets decreased by 5.1% in Kenya and by 8% in Ethiopia. Moreover, improvement of crop residues decreased annual Ei by 27.5% in Kenya and by 30.5% in Ethiopia. Our findings suggest that urea treatment of crop residues is an accessible strategy for reducing CH4 emissions intensity in East African dairy production systems.

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Ivermectin-treated birdfeed confers dose-dependent toxicity to *Culex tarsalis* mosquitoes

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West Nile Virus transmission is sustained in an enzootic cycle between ornithophilic *Culex* species mosquitoes and birds, highlighting this relationship as a potential target for intervention. Specifically, we can expose ivermectin (IVM) to *Culex tarsalis* via bloodmeals from birds treated with IVM-treated feed. IVM binds to glutamate-gated chloride channels (GluClR), causing paralysis and death in invertebrates while maintaining a robust safety profile in vertebrates due to differences in GluClR localization between species. To investigate this method, we provided chickens with IVM-treated feed at doses of 200 mg IVM per kg of feed (mg/kg IVM) and 360 mg/kg IVM for up to 7 days. We collected sera via jugular venipuncture on days 3 and 7 of the diet, then reconstituted it with chicken red blood cells to provide bloodmeals to 3-6 day old female laboratory-raised *Culex tarsalis* mosquitoes. Additionally, we observed the birds' behavior to determine whether IVM caused any adverse effects during or following treatment. Our results demonstrate dose-dependent toxicity in *Culex tarsalis* mosquitoes with Mantel-Haenszel Hazard Ratios of 3.150 at day 3 of a 200 mg/kg IVM diet (IVM n=65, control n=63, P value<0.0001) and 7.068 at day 7 of the same diet (IVM n=58, control n=42, P value<0.0001), compared to 26.41 at day 3 of a 360 mg/kg IVM diet (IVM n=50, control n=84, P value<0.0001) and 28.74 at day 7 of the same diet (IVM n=53, control n=69, P value<0.0001), showing a pattern of decreased probability of survival with increased dose and feed consumption. Treated birds experienced no adverse events and consumed similar amounts of feed compared to control birds. Analysis of 500 mg/kg IVM diet results, IVM concentration in sera, and histopathology is ongoing, and experiments with wild-type *Culex* species mosquitoes and wild-caught birds are planned for the upcoming field season. Results from these experiments will inform future decisions regarding the safest and most efficacious dose and formulation of IVM-treated feed to proceed to field trials.

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Investigating the function of RAD51AP1 in homologous recombination DNA repair

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DNA repair processes are crucial for mending DNA damage, maintaining genome integrity, and preventing mutations leading to cancer. One major DNA repair process is homologous recombination DNA repair (HR), a process that requires the formation of the presynaptic RAD51 nucleoprotein filament to align homologous DNA sequences and initiate repair. RAD51-Associated Protein 1 (RAD51AP1) enhances RAD51 activity, yet how exactly RAD51AP1 functions during HR is still poorly understood. Our main objective is to define key attributes of RAD51AP1 by further investigating its DNA-binding properties. Using recombinant human RAD51AP1 in the electrophoretic mobility shift assay, we find that RAD51AP1 avidly associates with both naked and chromatinized double-stranded (ds)DNA. Deletional and mutational analyses were used to further define these associations. We find that the N-terminal 94 residues of RAD51AP1, which associates with naked dsDNA, are devoid of binding to chromatinized dsDNA. In contrast, the C-terminal 148 residues of RAD51AP1 show affinity for both naked and chromatinized dsDNA. Two post-translational modification sites within its C-terminal DNA-binding region were also evaluated and showed decreased affinity to chromatinized dsDNA. Based on these findings and other results, we propose a model in which RAD51AP1 guides homology search and hetero duplex formation in the HR reaction.

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Pathogen interactions in polymicrobial *Escherichia coli* and *Enterococcus faecalis* extraintestinal infections

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Extraintestinal pathogens *E. coli* (ExPEC) and *Enterococcus faecalis* (EF) escape the intestinal niche and colonize extraintestinal tissues. Co-infections of these two pathogens result in debilitating polymicrobial urinary tract and bloodstream infections in people and animals. Factors contributing to disease occurrence, severity, and resolution include bacterial genetics, interactions between ExPEC and EF, and the host response. Unraveling the drivers of ExPEC/EF polymicrobial infection requires a multifaceted approach. By chelating iron, an essential nutrient for ExPEC, an *in vitro* co-culture assay was developed to mimic the iron limitation in the extraintestinal environment. This model allowed us to screen a genetically-diverse ExPEC collection and identify strains that could overcome low iron in response to diffusible EF signals. Then, an *in silico* comparative genomics approach implicated iron scavenging siderophores, protective polysaccharide capsule, and conjugative pilin as potential drivers of ExPEC growth induction by EF. The co-culture model was expanded to include RNA-sequencing and capsule quantitation, which demonstrated some of these features are overexpressed or overproduced by ExPEC in response to EF. Finally, an *in vivo* embryo infection model quantified the virulence of ExPEC and EF co-infections. ExPEC strains that were unresponsive to EF signals *in vitro* were attenuated during co-infection *in vivo*. Thus, our approach revealed a complex induction mechanism requiring several coordinated steps in ExPEC as they respond to EF. This interaction requires further characterization using a variety of methods so that therapeutic approaches to disrupt it can be employed to treat ExPEC/EF polymicrobial infections.

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