2nd National Colloquium for Combined DVM/PhD Biomedical Scientists

August 1-2, 2018

College of Veterinary Medicine & Biomedical Sciences
Texas A&M University

Host Organizer

Roger Smith, D.V.M., Ph.D.
Texas A&M University

Session Chairs

Michael Atchison, PhD
University of Pennsylvania

Xinbin Chen, BVSc, PhD
University of California, Davis

Edward Hoover, DVM, PhD
Colorado State University

Hélène Marquis, DVM, PhD
Cornell University

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We sincerely appreciate the support of our sponsors. Due to their generosity, we were able to invite all combined degree students, without charging a registration fee.

COLLEGE OF VETERINARY MEDICINE
& BIOMEDICAL SCIENCES
Department of Veterinary Pathobiology

TEXAS A&M UNIVERSITY

August 1, 2018

Dear Friends & Colleagues:

Howdy and welcome to the 2nd National Colloquium for Combined DVM/PhD Biomedical Scientists at the Texas A&M College of Veterinary Medicine & Biomedical Sciences. The Colloquium had its origin at the National Veterinary Scholars Symposium, where dedicated veterinary educators came together to create an exchange of ideas and best practices for training veterinary scientists. From a meeting of several faculty members, it grew into the first Colloquium, hosted last year by Dr. Mark Simpson at the National Institutes of Health. We are honored to be hosting the second Colloquium at Texas A&M.

As this expanded to a Colloquium, we had the opportunity to feature some of our outstanding combined degree students by inviting a few of them to present their scientific findings. Everyone found it exciting to hear their impressive work, and this year we were able to include poster presentations by nearly all of our combined degree students, in addition to talks. The students are also continuing to develop networks among the different programs. With their valuable input, we have invited a panel of recent combined degree graduates to sit on a panel focused on careers and life after the dual degrees. It will be exciting to see where this fledgling Colloquium goes in the next few years.

Veterinary clinician scientists are an important component of the nation's research effort on so many fronts: human, animal and environmental health, therapeutic discoveries, fundamental discovery science, and more. But the veterinary community's scientific contributions can become even greater. It is incumbent upon veterinary curricula and faculty members to encourage the research track for our students, to nurture those with research interests and scientific curiosity, and to provide strong training programs that allow them to succeed. Combined degree programs are one of several paths to success as veterinary clinician scientists and are certainly the best path for many students.

The clinical training that veterinary students receive provide them the tools and understanding of health and disease, both in individuals and in populations. The training is multi-disciplinary: physiology, pathology, immunology, microbiology, parasitology, pharmacology, public health. Students are exposed to the whole of comparative medicine and surgery. They graduate equipped to engage scientists across disciplines, engagement that is essential in today's scientific research effort.

This Colloquium celebrates the quality of our students and our training programs to excel in multi-disciplinary, collaborative and comparative biomedical research. We welcome you to Aggieland and wish you a great Colloquium.

Wishing you the best,

Roger Smith III

Roger Smith III, DVM, PhD
Professor, Department of Veterinary Pathobiology
College of Veterinary Medicine & Biomedical Sciences
Texas A&M University

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Schedule of Events

Wednesday, August 1		
7:00 PM	VENI 101	Informal Gathering & Buffet Dinner (cash bar)
Thursday, August 2		
7:00 AM	VENI 101	Breakfast
8:30 AM	VENI 106A	Dr. Roger Smith – Welcome Address and House-Keeping Issues
8:45 AM	VENI 106A	Katti Horng, University of California, Davis
9:00 AM	VENI 106A	Kristen Davenport, Colorado State University
9:15 AM	VENI 106A	Emily Mackey, North Carolina State University
9:30 AM	VENI 106A	Emily Pope, University of Minnesota
9:45 AM		Break
10:15 AM	VENI 106A	Dr. Stephanie Murphy — by videoconference Director of the Division of Comparative Medicine Office of Research Infrastructure Programs Division of Program Coordination, Planning, and Strategic Initiatives Office of the Director, National Institutes of Health
10:45 AM	VENI 106A	Frances Chen, Cornell University
11:00 AM	VENI 106A	Elinor Willis, University of Pennsylvania
11:15AM:	VENI 106A	Col. Jennifer M. Kishimori Office of the Assistant Secretary Defense for Health Affairs Health Protection Readiness and Oversight Director, Chemical, Biological, Radiological and Nuclear Medical Countermeasures Policy
11:45 AM	VENI 101	Lunch
1:15 PM	VENI 106A	Panel discussions with Dr. Theresa Alenghat Dr. Erin Chu Dr. Tim Kurt Dr. Roxann Brooks Motroni Dr. Noelle Noyes
3:45 PM	VENI Foyer	Poster Session & Afternoon Refreshment Break
4:15 PM	VENI 106A	Dr. Harold Watson — T-Directors Meeting (videoconference)
5:30 PM	VENI 106A	Closing remarks

Speaker Biographies

Stephanie Murphy, VMD, PhD

Director of the Division of Comparative Medicine
Office of Research Infrastructure Programs
Division of Program Coordination, Planning, and Strategic Initiatives
Office of the Director
National Institutes of Health

Dr. Murphy received both her V.M.D. and Ph.D. from the University of Pennsylvania and completed a comparative medicine postdoctoral fellowship at The Johns Hopkins University School of Medicine, along with all of the requirements to gain the status of Diplomate of the American College of Laboratory Animal Medicine. Dr. Murphy was named the Director of the Division of Comparative Medicine (DCM), Office of Research Infrastructure Programs (ORIP) in June 2014. DCM's mission is to develop veterinary scientists as part of the workforce that supports and contributes to animal-based research and resources as well as to support biomedical research in the form of diverse models of human disease using vertebrate and non-vertebrate animals or cultured cells. She joined ORIP from the Oregon Health and Science University (OHSU), where she was a Professor of Anesthesiology and Perioperative Medicine (APOM), with joint appointments in Behavioral Neuroscience and Comparative Medicine. Dr. Murphy has published numerous articles, reviews and book chapters related to her research and clinical interests regarding sex differences and the role of sex steroids in stroke as well as development and management of animal models related to stroke and women's health.

Col. Jennifer M. Kishimori, DVM, PhD

US Army Veterinary Corps
Director, Chemical, Biological, Radiological and Nuclear (CBRN) Medical Countermeasures Policy
Office of the Assistant Secretary Defense for Health Affairs (OASD(HA))
Health Protection Readiness and Oversight (HRP&O)

Colonel (COL) Jennifer M. Kishimori serves as the Director, Chemical, Biological, Radiological and Nuclear (CBRN) Medical Countermeasures Policy in the Office of the Assistant Secretary of Defense for Health Affairs, Health Readiness Policy and Oversight. In this role, she leads DoD health policy development and oversight efforts in CBRN medical product research and development, coordinating within DoD Components and across the Interagency to ensure readiness of U.S. Forces against the effects of weapons of mass destruction. COL Kishimori also serves as the Veterinary Biomedical Scientist (D.V.M./Ph.D.) (Military Occupational Specialty 64E) Consultant to the U.S. Army Surgeon General, and oversees the recruitment, professional development, and assignment of officers in this specialty.

COL Kishimori graduated in 1992 with a B.A. in Biology from the Johns Hopkins University and was commissioned a Second Lieutenant through the Army ROTC Program (Distinguished Military Graduate). She received a D.V.M. from the North Carolina State University College of Veterinary Medicine in 2003, and a Ph.D. in Microbiology from the University of Hawaii in 2010 through the U.S. Army's Long Term Health Education and Training Program. COL Kishimori previously served in the U.S. Army as a Military Intelligence Officer in Korea and in the 101st Airborne Division (Air Assault), prior to completing veterinary school and entering the U.S. Army Veterinary Corps.

Over the past 15 years, COL Kishimori's service as a U.S. Army Veterinarian has ranged from veterinary clinical medicine and food protection missions at Fort Bragg, North Carolina and Yongsan Army Garrison, Seoul, Korea to military research, development and acquisition as a 64E Veterinary Biomedical Scientist. She has served as a bench researcher, Deputy Virology Division Chief and Military Deputy to the Scientific Director at the U.S. Army Medical Research Medical Research Institute of Infectious Diseases, supporting the research and development of medical countermeasures against select biological agents and toxins. She also served as Deputy Director and Director, Force Health Protection Division at the U.S. Army Medical Materiel Development Activity, where her team provided investigational medical countermeasure support to Operation United Assistance during the 2014 West Africa Ebola Outbreak Response.

COL Kishimori is a graduate of the U.S. Army's Command and General Staff Officers' Course and the Naval Post Graduate School's Advanced Acquisition Program. She is certified in Science and Technology Management (Level III) from the Defense Acquisition University and is a Project Management Professional.

Theresa Alenghat, VMD, PhD

Assistant Professor
Department of Pediatrics
University of Cincinnati

Dr. Theresa Alenghat is an Assistant Professor in the Immunobiology Division at Cincinnati Children's Hospital Medical Center. Dr. Alenghat received her veterinary degree and pathology residency training at the University of Pennsylvania. She also did her PhD in molecular biology as well as postdoctoral work at the same institution working with Drs. Mitch Lazar and David Artis, leaders in the fields of metabolism and immunology, respectively. She joined Cincinnati Children's in 2014 and has established a research program to investigate molecular mechanisms that regulate the host-microbe relationship and how this level of regulation affects susceptibility to infection, inflammatory bowel disease, and obesity. Dr. Alenghat oversees a Gnotobiotic Mouse Facility and play an active role in the Immunology, Cell Biology, and Developmental Biology Graduate Groups and the Medical Scientist Training Program. Her research includes investigation of epigenomic pathways that regulate epithelial and immune cell homeostasis in the context of the signals from the intestinal microbiota. Her work in this area has been published in multiple high profile journals including Nature, Science, Nature Immunology, and Immunity. At this early stage, Dr. Alenghat has also already been successful in securing substantial extramural funding from the NIH, Burroughs Wellcome Fund, Pew Charitable Trust, and the Crohn's & Colitis Foundation.

Erin Chu, DVM, PhD

Senior Veterinary Geneticist
Embark Veterinary, Inc

Erin graduated from Cornell's Combined DVM/PhD program in 2017, having tracked equine in veterinary school, then pursuing her PhD in the lab of Dr. Paul Soloway with a focus on long noncoding RNA-mediated epigenetic mechanisms using the mouse model. Throughout her PhD, Erin continued to practice with local high-quality, high-volume spay neuter and wellness programs. She also began consulting with Embark, a then seed-grant stage Cornell-affiliated startup. After graduating in 2017, she accepted on full-time offer with Embark in the versatile position of Senior Veterinary Geneticist. Within Embark, Erin acts the liaison between Embark's Research and Development and Science and Marketing spheres. She is heavily involved in product design and development, and helps to shape research studies and collaborations with breed clubs and rescue organizations. Erin is particularly interested in scientific and medical outreach and communication, especially as it applies to web-based data collection and citizen science, and owes much of her daily motivation and success to her Labrador-Shepherd mix, Shiloh.

Tim Kurt, DVM, PhD

Scientific Program Director
Foundation for Food and Agriculture Research

Dr. Tim Kurt establishes important collaborations between industry, academia, non-profits and other partners to address critical challenges facing agriculture. Dr. Kurt develops projects in FFAR's Protein Challenge and Rapid Outcomes from Agriculture Research programs at FFAR, and his portfolio includes research that ranges from the basic biological sciences to applied technologies in animal health and wellbeing, disease surveillance and response, genetics and sustainability. He is passionate about using science to advance the wellbeing of society.

Dr. Kurt received his DVM and PhD degrees from the combined degree program at Colorado State University, where he studied spongiform encephalopathies (prions) such as BSE/mad cow, scrapie and CWD – diseases which can be transmitted between livestock, wildlife and humans. Prior to joining FFAR in 2016, he worked as a Research Scientist at the Center for Veterinary Sciences and Comparative Medicine at the University of California San Diego (UCSD), where he received awards from the NIH, the Morris Animal Foundation and the AVMA/AVMF.

Roxann Brooks Motroni, DVM, PhD

National Program Leader for Animal Health
USDA Agricultural Research Station

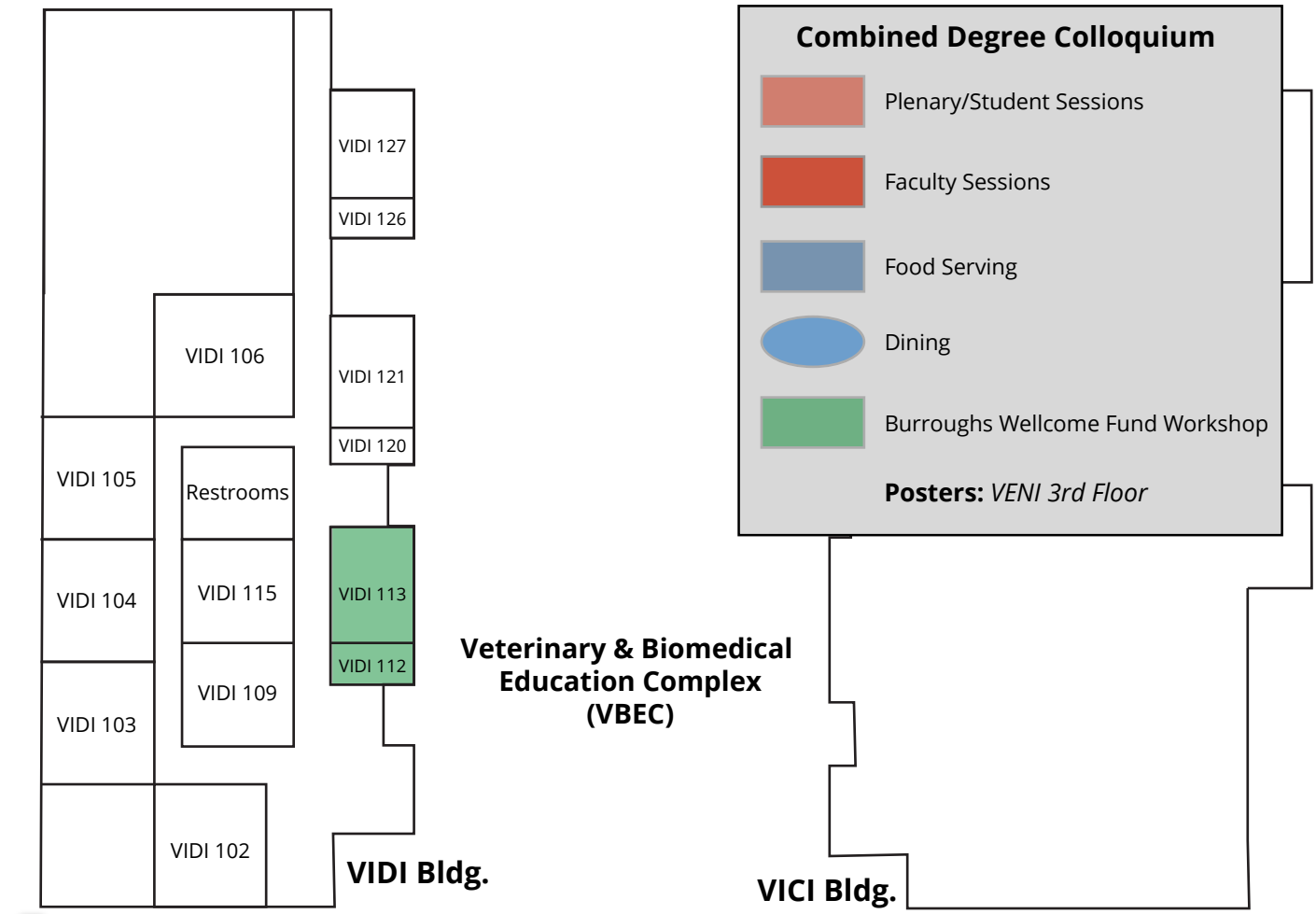
Roxann Brooks Motroni is the National Program Leader for Animal Health at the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). In this role, she sets the strategic direction and national coordination for USDA's intramural research program focused on animal health research which includes some zoonotic diseases with 9 research locations in Ames, Iowa; Clay Center, Nebraska; Athens, Georgia; Orient Point; New York, Beltsville, Maryland; Pullman, Washington; Mississippi State, Mississippi; Albany, CA and Manhattan, Kansas. Prior to this position, she was a program manager at the U.S. Department of Homeland Security (DHS), Chemical and Biological Defense Division (CBD) in the Agriculture Defense Branch. Her program funded between $10-15M per annum to provide discovery, early development, test and evaluation of countermeasures for foreign animal diseases that could have catastrophic impacts on the U.S. economy. She holds a Doctorate of Veterinary Medicine and a PhD in Comparative Pathology from the University of California, Davis and is a practicing food animal veterinarian.

Noelle Noyes, DVM, PhD

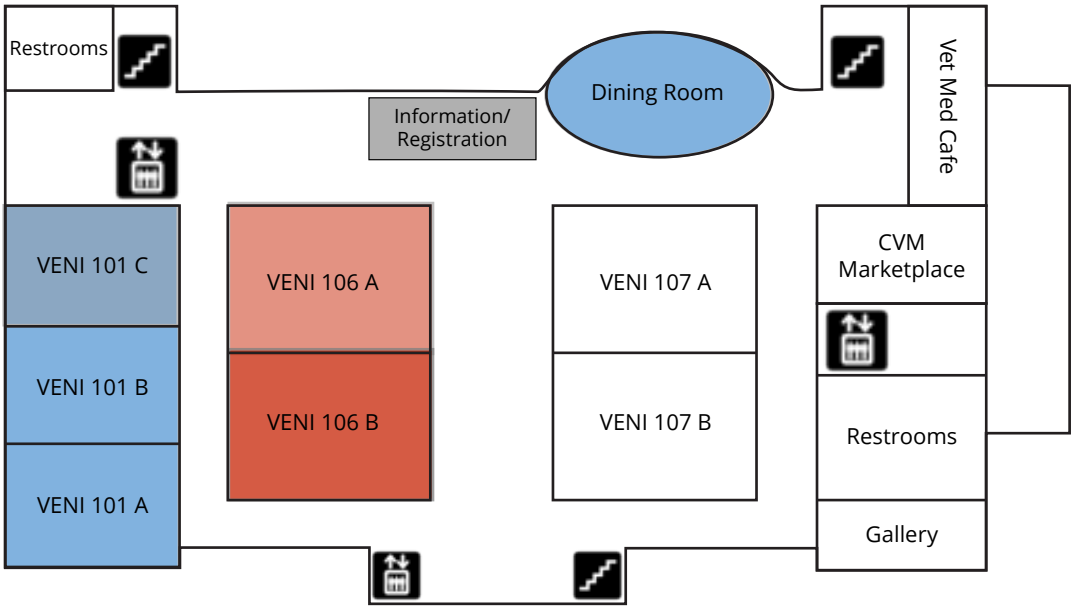
Assistant Professor
Department of Veterinary Population Medicine
University of Minnesota

Dr. Noelle Noyes is a veterinary epidemiologist in the Veterinary Population Medicine Department at the University of Minnesota. Noelle received her BA in European Studies from Amherst College, with a secondary concentration in Asian Languages and Civilizations. She received her MA from Osnabrück Universität (Germany) while conducting independent research on a German Chancellor Fellowship from the Alexander von Humboldt Foundation, and bartending at the local kneipe. Her Master's thesis investigated the ethnological context of immigration and integration of ethnic Germans within eastern and western Germany. She then worked as a consultant for Mercator Partners in Boston, specializing in innovation strategy and mergers/acquisitions for high-tech companies, including the Sprint-Nextel merger. After deciding that corporate American wasn't for her, Noelle decided to pursue veterinary school. While waitressing and wrangling cows, dogs and cats (not at the same time), Noelle took all of the science pre-reqs for vet school and was accepted into the DVM-PhD program at Colorado State University, where she received her doctorate in epidemiology, a USDA NIFA post-doctoral fellowship, and her veterinary degree (large animal medicine). Noelle's current research program focuses on advancing our understanding of antimicrobial resistance ecology and epidemiology within livestock production systems, and at the livestock-human interface. To achieve this, her lab uses both traditional and nascent methodologies, in collaboration with scientists from diverse fields including veterinary medicine, epidemiology, statistics, animal science, computer science and molecular biology. Noelle also maintains an active outreach program, and strives to conduct applied research in close partnership with stakeholders in agriculture, veterinary medicine and government.

MAP

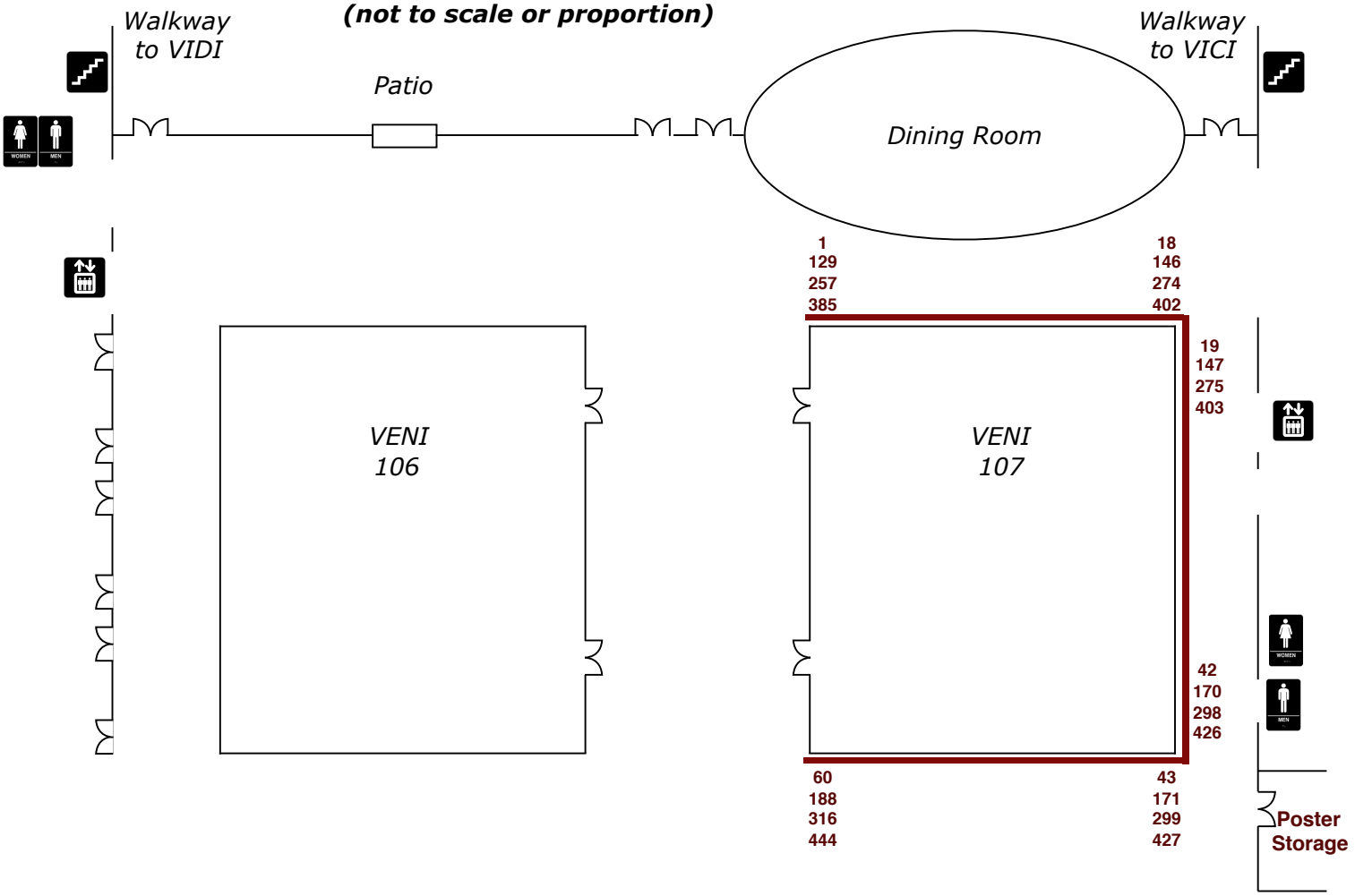


Shuttle Bus Stop



VENI Bldg. (1st Floor)

Posters
VENI 1st Floor
(not to scale or proportion)



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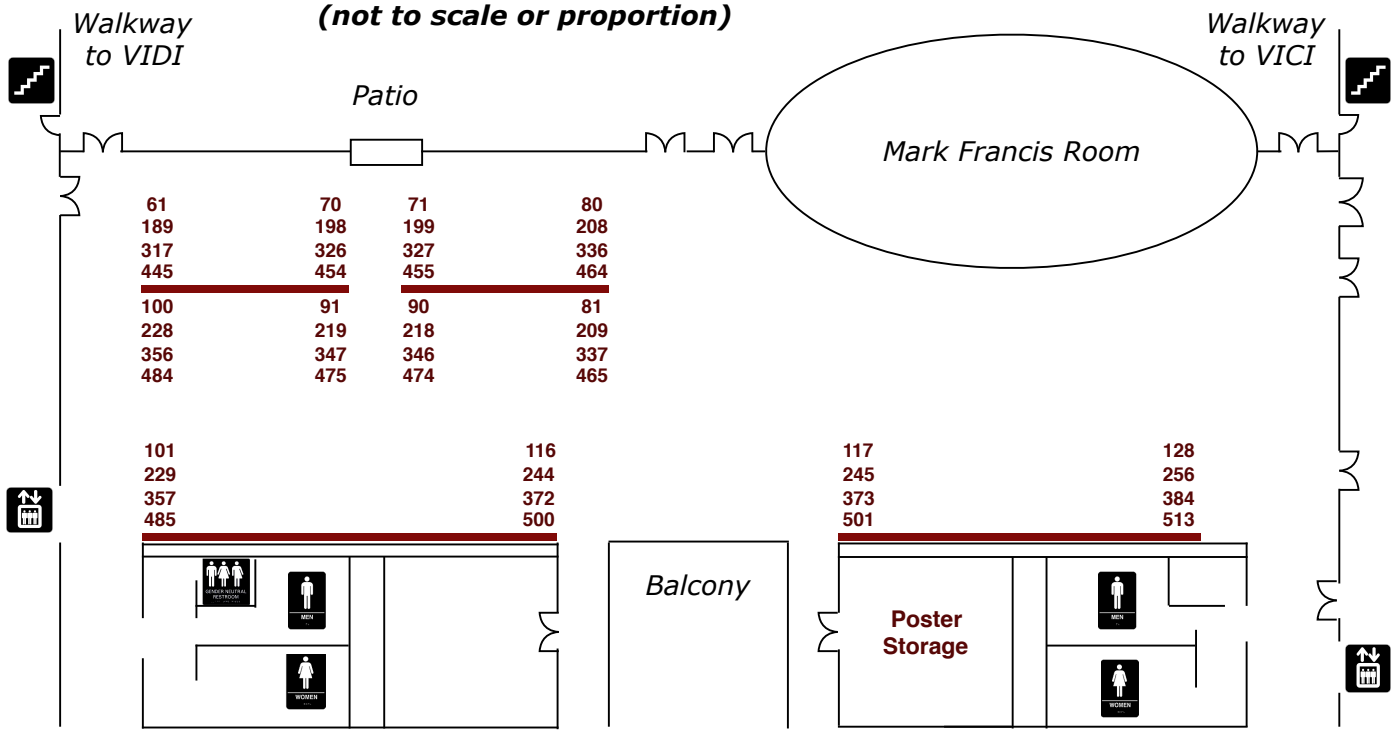
VENI 3rd Floor
(not to scale or proportion)



Student Abstracts

Posters presentations will be on the first floor, encircling the classroom opposite VENI 106A.
We will start with poster position 7 to provide some distance from the registration table.

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Poster #7

A novel effect of PD-L1 immune checkpoint molecule.

Dylan Ammons[1], Gen Hartley[1], David Ackart[2], Steven Dow[1]

[1]Animal Cancer Center, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Ft. Collins, CO
[2]Department of Microbiology, Immunology and Pathology, Colorado State University, Ft. Collins, CO

In recent years immune checkpoint blockade has become a promising cancer immunotherapy. This therapy aims to block inhibitory receptor-ligand interactions between T cells and other cellular counterparts in the tumor microenvironment (TME). By preventing receptor-ligand ligation T cells do not receive inhibitory signals and are able to remain active. One of the major blocking targets is program death ligand-1 (PD-1) and the commonly accepted belief is that blocking the interaction by targeting the ligand (PD-L1) or receptor (PD-1) would have the same result. Here we show that this may not be the case for PD-1/PD-L1 blockade and provide data which indicate PD-L1 blocking antibodies (αPD-L1 mAb) are able to activate murine bone-marrow derived macrophages. Treatment with αPD-L1 mAb induces morphologic and phenotypic changes which support the notion that the treated macrophages become activated and proliferate. We then identified that the changes are mediated via mTOR/AKT signaling axis. With evidence that signaling is mTOR mediated, macrophage metabolic changes were examined via extracellular flux analysis to better understand the functional changes induced by αPD-L1 mAb treatment. Data obtained though flux analysis indicated that treatment causes macrophages to become more metabolically active. Through flux analysis we noted that the response to treatment is altered by the presence or absence of the macrophage differentiation factor, M-CSF. The M-CSF/PD-L1 interaction was then examined through signal transduction and flux analysis to further elucidate the effect. Overall, this work provides valuable insight into additional functions of αPD-L1 mAb treatment which could contribute to different *in vivo* efficacies.
Supported by the Shipley Foundation and NIH graduate student stipend

Poster #8

From field to lab: lack of endogenous feline leukemia virus may leave pumas vulnerable to FeLV infection

Elliott Chiu, Mark Cunningham, Lara Cusack, Melody Roelke, Sue VandeWoude

Department of Micobiology, Colorado State University, Fort Collins, CO (Chiu, VandeWoude)
Florida Fish and Wildlife Conservation Commission, FL (Cunningham, Cusack)
National Institutes of Health, MD (Roelke)

Feline leukemia virus (FeLV) regularly infects domestic cats and has been frequently documented to spill over to many wild felid species with devastating clinical outcomes. While cats harbor a near complete endogenized FeLV (enFeLV) resembling the infectious, horizontally transmitted virus, many other felid species, lack enFeLV. EnFeLV recombination is responsible for recombinant oncogenic FeLV variants such as FeLV-B; it is also believed to block horizontal transmission and partially protect against FeLV disease. We thus hypothesize that enFeLV plays a role in intrinsic FeLV restriction in domestic cats and lack of enFeLV correlates with more virulent disease in other felids. Our studies have documented the first detection of a FeLV-B in a non-domestic felid, and have demonstrated multiple cross species transmissions in free ranging Florida panthers (Puma concolor coryi). We measured proviral load, viral replication, and viral antigen production following FeLV infection of domestic cat and puma fibroblasts and peripheral blood mononuclear cells to evaluate the capacity of FeLV to replicate in different felid cells. Domestic cat cells displayed a negative correlation between FeLV replication and enFeLV copy number, dependent upon enFeLV LTR versus *env* segments. Interestingly, puma cells supported significantly greater viral replication. Differential expression of enFeLV in various tissues indicates that lymphoid tissues, the primary replication site of FeLV, express enFeLV greater transcription than other tissues. In sum, the FeLV system presents an opportunity to examine endogenous-exogenous retroviral interactions from lab bench to natural populations, providing insight into endogenous retrovirus biology in humans.

Research Grants
NSF EID 1413925
Winn new feline investigator award W18-018

Student Support
NIH F30 OD0827386
NIH T32 OD012201
CSU DVM/PhD program

Poster #9

β-endorphin from proopiomelanocortin neurons can mediate activity-based anorexia in mice

Caitlin M Daimon, Christina S Dennison, Shane T Hentges

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

Proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus potently inhibit food intake and their over-activation may contribute to the development of anorexia. Activity-based anorexia (ABA) is a commonly used rodent model of anorexia (reduced feeding) in which timed food presentation paired with availability of a running wheel results in reduced food intake, increased wheel running activity and bodyweight loss. However, it is unknown whether inhibiting POMC neurons and the subsequent release of their peptide products would be sufficient to reduce the development of ABA. To examine the possibility that inhibiting POMC neurons could lessen the development of ABA, chemogenetic (Designer Receptors Exclusively Activated by Designer Drugs; DREADD) technology was used to selectively inhibit POMC neurons. Consistent with our hypothesis, inhibiting POMC neurons led to a significant decrease in food anticipatory activity (enhanced running before food presentation), a hallmark of the ABA model. To determine if the development of food anticipatory behavior in mice requires the release of the POMC peptide β-endorphin, we tested how the administration of an antagonist of the receptors which β-endorphin acts on affects the development of ABA. We found a significant decrease in food anticipatory activity when the antagonist naloxone was administered. Bodyweight loss was less severe when POMC neurons were inhibited and also when naloxone was administered compared to saline-treated animals. Taken together, the results suggest that inhibiting POMC neurons results in a less severe presentation of ABA and that this is mediated, at least in part, by β-endorphin.

Research grant: R01DK078749 to STH

Student support: F30DK117530 to CMD

Poster #10

Prion shedding in saliva explains the horizontal transmission of chronic wasting disease

Kristen A. Davenport[1], Clare E. Hoover[1], Brittany A. Mosher[2], Brian M. Brost[2], Davin M. Henderson[1], Nathaniel D. Denkers[1], Candace K. Mathiason[1], Edward A. Hoover[1]

[1]Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO
[2]Department of Fish, Wildlife and Conservation Biology, College of Natural Resources, Colorado State University, Fort Collins, CO

Chronic wasting disease (CWD), a neurodegenerative disease of cervids, is unique among prion diseases in its efficient horizontal transmission in wild populations. CWD is spreading across North America and has reached Asia and, recently, Scandinavia. Prions have been detected in tissues and excreta of CWD-infected cervids, but the course prions take from initial infection to excretion and the magnitude and frequency of prion shedding remain unknown. To answer these questions, we: (1) used *in vitro* amplification methods to compare the distribution of prions in lymphoid and gastrointestinal tissues of deer early and late in disease, and correlated prion distribution with prion shedding in saliva and neuroinvasion; (2) used an ecology modeling approach to demonstrate that the likelihood of prion shedding in saliva was 2.77 times higher in deer inoculated with saliva vs. brain; (3) demonstrated that deer saliva commonly contained an inhibitor of the real-time, quaking-induced conversion (RT-QuIC) assay and characterized the inhibitor as a member of the mucin family; and (4) developed an alternate protocol to bypass the inhibitor and improve detection of prions in saliva. Taken together, our data demonstrate that prions are shed in saliva earlier and more frequently than previously expected, and suggest that saliva plays an important role in the larger story of facile horizontal spread of CWD among cervids.

Research Support: NIH N01AI25491, NIH R01NS047433, and NIH R01NS061902

Student Support: NIH F30OD021442

Poster #11

Antimicrobial use in beef feedlots; effects on microbiome and resistome dynamics

Authors:
E. Doster , J. K. Parker, C. A. Anderson, S. M. Lakin, N. R. Noyes, M. Weinroth, C. W. Booker, S. J. Hannon, S. P. Gow, T. A. McAllister, K. E. Belk, P. S. Morley.

Affiliations:
Department of Clinical Sciences (Parker, Anderson, Morley), Animal Sciences (Weinroth, Belk), and the Department of Microbiology, Immunology and Pathology (Doster, Lakin), Colorado State University, Fort Collins, Colorado; Department of Veterinary Population Medicine, University of Minnesota, St. Paul, Minnesota (Noyes); Feedlot Health Management Services, Ltd., Okotoks, Alberta T1S 2A2, Canada (Booker, Hannon); Centre for Food-borne, Environmental Zoonotic Infectious Diseases, Public Health Agency of Canada, University of Saskatoon, Saskatchewan, Canada. (Gow); Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB, Canada. (McAllister)

Abstract Content:
The increase of antimicrobial resistance (AMR) in pathogens is a global public health concern and is commonly hypothesized to be “driven” by antimicrobial use (AMU) in health care and livestock production. Therefore, it is important to understand how AMU practices in beef feedlot operations impact AMR dynamics in fecal bacteria that could spread to the surrounding environment. Traditionally, AMR research uses aerobic culture to study just a few bacterial species from a complex bacterial community (microbiome) and results can differ depending on the species under study. Fortunately, advancements in high-throughput sequencing can be used to provide a holistic perspective into AMR ecology by sequencing DNA from the entire microbiome, including the “profile” of resistance genes (resistome). In this study we employ metagenomic sequencing to characterize the effect of AMU on the microbiome and resistome in feces collected during a previously published 3-year longitudinal study of Canadian beef feedlot operations. Pens of cattle were randomly selected for inclusion into the study and pooled fecal samples were collected from the pen floor when cattle arrived to the feedlot and at a second date during the feeding period. All AMU, including parenteral treatments and in-feed exposures, was recorded and standardized using animal defined daily dose (ADD). Pen level AMU was calculated as the sum of ADDs for all cattle housed in a pen. Our preliminary results characterize the microbiome and resistome ecology in beef feedlot operations; further, we are able to make unique comparisons of results investigating the impact of AMU on AMR produced by traditional aerobic culture methods compared to the modern tool of metagenomic sequencing.

Research Grant: USDA NIFA grant #215-68003-2304

Student Support: NIH-T32 Predoctoral Scholar Program (Colorado State University)

Poster #12

'Ironing out' *Mycobacterium tuberculosis*-mediated changes in macrophage metabolism

Dilara Kiran[1], Andres Obregon-Henao[1], David Ackart[1], Michio Kuruso[2], Branch Moody[3], Brendan Podell[1], Randall Basaraba[1].

1. Department of Microbiology, Immunology, Pathology; College of Veterinary Medicine and Biomedical Sciences; Colorado State University; Fort Collins, CO
2. College of Pharmacy; University of Tennessee Health Science Center; Memphis, TN
3. Division of Rheumatology, Immunology, and Allergy; Brigham and Women's Hospital; Boston, MA

Lactate, the product of glycolysis, is a known substrate for oxidative metabolism and contributes to immunosuppression. During *Mycobacterium tuberculosis* (*Mtb*) infection, serum lactate levels are elevated and lactate concentrations within the lung granuloma increase. We have shown that guinea pigs infected with *Mtb* demonstrate increased plasma lactate levels when compared to uninfected animals, and that therapeutic intervention can lower these levels. Despite this knowledge, the function of lactate and mechanisms by which it accumulates during *Mtb* infection are unclear. Hypoxia inducible factor-1α (HIF-1α) upregulates glycolysis and increases lactate production. HIF-1α degradation requires iron cofactors and iron chelation has been shown to activate HIF-1α by inhibiting its degradation. We hypothesize that the iron-chelating siderophore produced by *Mtb*, mycobactin, is responsible for driving HIF-1α activation during early infection, facilitating a metabolic shift from oxidative metabolism to glycolysis and causing lactate accumulation. We have demonstrated that guinea pig bone marrow derived macrophages treated with mycobactin exhibit increased HIF-1α protein levels in a concentration-dependent manner. Mycobactin knock-out strains of *Mtb* will be used to determine whether mycobactin-mediated, hypoxia-independent HIF-1α activation drives a metabolic shift to glycolysis and subsequent lactate accumulation *in vitro* and *in vivo*. We will determine whether this mechanism leading to increased lactate production alters immune cell function and how accumulated lactate is utilized within the granuloma to drive the formation of an *Mtb* survival niche and prevent efficient *Mtb* clearance.

Research Grants:
1U19AI111224-01
1R21AI107254
1R01AI106733

Student Support:
CSU CVMBS Predoctoral T32 (July 2017-July 2018)
1 F30 OD024647-01A1 (awarded 07/03/2018)

Poster #13

Environmental persistence of MAP as a barrier to Johne's disease elimination

Kristina Ceres, Mohammad Abdullah Al-Mamun, Yrjö Gröhn

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Johne's disease is pervasive on dairy farms in the United States and is notoriously difficult to control. One suspected reason for the difficulty in managing Johne's disease is the ability for *Mycobacterium avium ssp. paratuberculosis* (MAP), the causative agent, to persist in the environment. Animals become infected with MAP by ingesting contaminated material in their environment, and once infected can shed large quantities of MAP in their feces. With fecal shedding coupled to the ability of MAP to persist in the environment, dairy farmers may be unable to rid their herd of Johne's disease without intense focus on environmental management. Although it is well documented that environmental contamination is an important factor in MAP transmission, few simulation models include an environmental component, and no agent-based model with explicit environmental transmission exists. We developed an agent-based model of Johne's disease in a US dairy farm with explicit environmental MAP transmission. Individual cows, grouped by age and production characteristics, could become infected through exposure to MAP in their simulated environment. Infected animals could contaminate their environment by shedding MAP quantities proportional to the amount of manure produced and their infection stage. Using this model, determined if herd level MAP elimination was possible through intensive environmental management. We also determined if a threshold of environmental MAP contamination exists that prevents elimination. We found that MAP elimination is possible with vigorous cleaning alone, but the level of hygiene necessary for elimination is likely impossible to achieve in a true herd.

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Poster #14

Genome editing at the AF-associated *Pitx2* Locus: a role for the lncRNA *Playrr* in Cardiac Arrhythmias

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Atrial Fibrillation (AF) is the most common arrhythmia worldwide in humans, with serious implications such as stroke, cardiac arrest, and death. The most significantly associated AF risk loci map to variants in a noncoding, putative regulatory gene desert upstream of the *Pitx2* locus. Indeed, loss of *Pitx2* in mice and humans is associated with congenital cardiac defects, increased susceptibility to atrial fibrillation (AF), development of bilateral sinoatrial nodes (SAN), and sinus node dysfunction (SND). However, the genetic mechanisms by which *Pitx2* and its associated *cis*-regulatory elements at the locus contribute to the development of AF remain unclear.

Previously we discovered *Playrr*, a conserved enhancer-associated long noncoding RNA (lncRNA), arising from the *Pitx2* locus gene desert. To investigate the role of *Playrr* in regulating *Pitx2*, we used CRISPR/Cas9 genome editing in mice to target the *Playrr* RNA transcript while leaving its associated enhancer element intact ($Playrr^{Ex1sj}$). By adapting an awake surface ECG device (AliveCor) for use in mice, we detected cardiac arrhythmias in $Playrr^{Ex1sj}$mutants and validated these results with telemetry ECG. We demonstrate that adult $Playrr^{Ex1sj}$ mutant mice exhibit bradycardia and irregular R-R intervals that are indicative of sinus node dysfunction (SND), a bradyarrhythmia and risk factor for the development of AF. Finally, programmed stimulation reveals that $Playrr^{Ex1sj}$ mutants are predisposed to pacing-induced AF, strikingly similar to *Pitx2* loss-of-function mutant heterozygous mice. Our data suggests that *Playrr* modulates *Pitx2* gene dosage in the heart leading to conduction abnormalities and predisposition to important cardiac arrhythmias.

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Poster #15

Novel cellular tools to study HIV replication in tissue reservoirs and improve standard viral outgrowth assays

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Human immunodeficiency virus (HIV) remains a dominant global health crisis, affecting tens of millions of people worldwide. Viral reservoirs are established early during acute infection, and despite years of fully suppressive antiretroviral therapy (ART), they persist for the lifetime of the patient in an unknown diversity of cells. Our prior work demonstrated the presence of HIV mRNA in tissue-resident alveolar macrophages in the lungs of Malawian adults in Malawi, Africa, suggesting these cells may be viral reservoirs. Aided by cell-level HIV readouts, we have recovered infectious HIV from bronchoalveolar lavage and are studying the human transcripts and viral adaptations that facilitate this persistence. New reporter cell lines we have developed have shown that standard protocols of viral outgrowth likely miss many replication competent viruses within the context of gold-standard assays, underestimating the true HIV burden during HIV or SIV cure or treatment interruption trials. Our findings suggest that rare HIV proviruses within tissue reservoirs may be poorly adapted to replicate in standardized, homogeneous outgrowth assays. Furthermore, comparing human monoctye-derived macrophages with other cells in the tissue-resident macrophage lineage, we have uncovered opportunities to pursue novel restriction factors that may affect HIV persistence in vivo. Our overarching objective is to contribute to new HIV therapies targeted to specific tissue reservoirs.

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Poster #16

Dorsal raphe serotonin neurons and emotional gain

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Serotonin (5-HT), a widely-projecting neuromodulatory system, has been implicated in a variety of behaviors and brain states. The development of a theoretical model of 5-HT function that accounts for this diversity has challenged the field. As a result, the mechanism by which 5-HT levels affect emotional brain states and behavior remains unclear, despite the common use of selective serotonin reuptake inhibitors (SSRIs) in the treatment of depression and anxiety disorders. We hypothesize that 5-HT amplifies current emotional states, intensifying the positive value of rewarding conditions, and the negative value of aversive conditions. Here, we describe two experiments to test this hypothesis using choice as a behavioral read-out. The first experiment is a conditioned place preference paradigm. Mice show slight preference for or avoidance of places with mildly rewarding or aversive stimuli, respectively. We predict that conditioning with optogenetic stimulation of dorsal raphe 5-HT neurons will increase preference for or avoidance of these places accordingly. The second experiment is an operant chamber task where mice choose between performing an action on the left or the right to collect a probabilistic reward. We predict that 5-HT neuron stimulation upon reward retrieval or omission on one side will shift the preference toward or away from that side, respectively. Current work is focused on task optimization and behavioral characterization. The results of these experiments will determine how stimulation of dorsal raphe 5-HT neurons affects reward valuation and choice, which will shed light on the role of this system in emotional gain and state-dependent behavioral regulation.

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Student Support: Cornell University (Melissa R. Warden)

Poster #17

Inducible *hGlp-1r* knockout in the mouse hypothalamus promotes feeding and body weight gain

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Obesity and its associated co-morbidities, such as type II diabetes mellitus, represent widespread and costly public health epidemics. The current lack of effective, long-lasting anti-obesity treatments necessitates a better understanding of the neurobiological systems that regulate feeding behavior and energy balance. Due to its established role in reducing food intake and promoting glycemic homeostasis, the glucagon-like peptide-1 (GLP-1) system has been a promising target for pharmaceutical interventions. However, the mechanisms through which this incretin hormone acts in the central nervous system remain unclear. Conditional knockout studies offer an elegant technique to investigate the effects of endogenous receptor signaling in a targeted tissue without compromising normal ontogenetic development. Using the Cre/loxP system, we generated an inducible, site-specific knockout model of the GLP-1 receptor (GLP-1R) in the mouse hypothalamus. Adult male C57BL/6 mice expressing floxed human *Glp-1r* (*hGlp-1r*) were maintained on a 60% high fat diet to achieve diet-induced obesity. Subsequently, mice were stereotaxically injected with a Cre-expressing or control adeno-associated virus (AAV-Cre / AAV-Ctrl). Viral knockout of hypothalamic *hGlp-1r* caused increased food intake and body weight gain throughout the 14 days post-injection (Final 24h food intake: AAV-Ctrl = 3.06 ± 2.16 g, AAV-Cre = 4.30 ± 3.04 g, $p = 0.02$; Final body weight: AAV-Ctrl = 27.20 ± 0.10 g, AAV-Cre = 32.89 ± 1.04 g, $p = 0.06$). Altogether, these data demonstrate that endogenous GLP-1R signaling in the mouse hypothalamus is necessary to regulate energy balance.

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Student Support: Cornell University College of Veterinary Medicine Veterinary Investigator Program

Poster #18

Investigating the effect of p53 loss on folate metabolism in the development of neural tube defects

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Neural tube defects (NTDs) are birth defects that result in herniation and exposure of nervous tissue during embryogenesis when the neural tube fails to close. Folate deficiency causes failure of neural tube closure and it is estimated that 70% of all NTDs are folate responsive. However, the mechanism of this rescue effect remains to be elucidated. The tumor suppressor protein p53 plays an important role in development and both increased and decreased expression and/or function can lead to NTDs in mouse models. Studies *in vitro* demonstrate interactions among p53 and folate one-carbon metabolism pathways, specifically the *de novo* thymidylate (dTMP) biosynthesis pathway. Mouse models with impaired *de novo* dTMP synthesis have increased rates of NTDs that are responsive to folate supplementation. The aim of this project was to determine if folate supplementation could rescue $p53^{-/-}$ induced NTDs. Interestingly, $p53^{-/-}$ mouse embryonic fibroblasts have higher rates of folate-dependent *de novo* dTMP synthesis, purine biosynthesis, uracil accumulation in DNA, and proliferation. These results suggest that loss of p53 causes increased growth and thus increased nucleotide synthesis to accommodate, however not at levels that are sufficient to ameliorate uracil accumulation in DNA. Folate supplementation is also not adequate to prevent $p53^{-/-}$ NTD incidence.

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Poster #19

Mammary stem-like cells from mammary cancer resistant and susceptible species differ in response to UV

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Different species vary greatly in their mammary cancer incidence. Like humans, mammary tumors are among the most common neoplasms in intact female dogs and cats. Conversely, cows, mares, and sows rarely develop mammary tumors. Mammary stem cells (MaSC) reside in the adult mammary glands where they maintain and regenerate the tissue. As targets for malignant transformation, MaSCs may also be cancer cells of origin. Mammosphere-derived epithelial cells (MDEC) are primary mammary cells with stem-like properties that can be isolated from diverse species and used for comparative studies. We used MDEC to compare sensitivity to DNA damage between mammary cancer resistant and susceptible species. Previous work showed that MDEC from resistant species are more growth-sensitive to DNA damage (induced by DMBA or UV) than MDEC from susceptible species. RNA sequencing and qPCR analyses revealed that horse MDEC strongly upregulate p21 and downregulate Runx1T1 in response to DMBA or UV, whereas dog MDEC do not. p21 promotes cell cycle arrest in response to various stresses, including DNA damage. The function of Runx1T1 in the context of DNA damage is less known. Using qPCR, p21 and Runx1T1 expression was analyzed following UV exposure in MDEC from additional species that are mammary cancer susceptible (human and cat) or resistant (cow and pig). We did not observe an expression pattern of these genes that is unique to resistant or susceptible species. Upcoming experiments will be aimed at identifying the mechanism by which p21 is upregulated, the temporal pattern of p21 expression following UV exposure as well as investigating the role of p21 in the UV sensitivity of MDEC from mammary cancer-resistant species.

Research Grant: None
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Poster #20

Effect of diet on glycogen repletion in Thoroughbred horses after exercise

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When muscle glycogen stores in horses are depleted after exercise, re-synthesis of glycogen proceeds at a rate 2-3 times slower than that of other mammals. The reason for slow repletion is unknown. Humans and other mammals can speed glycogen replenishment by consuming carbohydrates after exercise, however, similar dietary manipulations to enhance repletion rates in horses has met with little success. We hypothesize that transcriptomic (RNAseq) and proteomic analysis of equine muscle during glycogen depletion and repletion will reveal the underlying mechanism responsible for slow glycogen repletion in horses. A cross over glycogen depletion and repletion trial using isocaloric high starch (HS) and low starch-high fat (LS) diets was performed in partnership with Kentucky Equine Research. HS and LS diets were fed to trained Thoroughbred horses for 3 weeks which was followed by 3 days of intense, glycogen depleting exercise and 3 days of repletion. Percutaneous gluteus medius muscle biopsies were obtained before and after exercise and during repletion. Muscle glycogen concentrations were measured fluorometrically as glucose residues. Muscle glycogen was depleted 67% (SD 17%) following exercise on HS and 70% (SD 8%) following LS diets. On Day 3 of repletion, muscle glycogen was 94% (SD 14%) repleted on HS, which was significantly greater than 63% (SD 20%) repletion for LS. Analysis of alterations in the skeletal muscle transcriptome and proteome during depletion and repletion is underway. Elucidating the underlying mechanism limiting glycogen repletion rate in horses may lead to nutritional methods to improve performance.
Research Grant: Michigan Alliance for Animal Agriculture and Kentucky Equine Research.
Student Support: Boehringer Ingelheim and the Michigan State University graduate school.

Poster #21

Oxylipid profiles of dairy cattle vary throughout the transition into early mammary gland involution

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Successful lactation in multiparous dairy cattle relies on a well-managed dry period that allows the mammary gland to remodel and regenerate between lactations. Oxylipids are potent inflammatory mediators that are capable of regulating all aspects of inflammation. Although an oxylipid profile has been documented for periparturient and lactating cattle, little work has been done to define the profile of cows in the early dry period. Therefore, our group aimed to characterize the oxylipid profile in healthy cows during the transition into early mammary gland involution. Plasma samples from 10 healthy Holstein dairy cows were collected via coccygeal venipuncture at D-6, D0, D+1, D+2, D+6, and D+12 relative to dry-off date. Liquid chromatography-mass spectrometry (LC-MS) was utilized to quantify select monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and saturated fatty acids while liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify oxylipids. The results of this study revealed a unique profile of pro- and anti-inflammatory oxylipids throughout the transition into the dry period. Many compounds reached highest concentrations of the study either at D+1 or D+12, which may be related to the physiological processes that are associated with the transition from a lactating to non-lactating state. The characterization of this profile allows for a basic understanding on how the oxylipids present during early involution may impact the health and productivity of dairy cows.

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Michigan State University College of Veterinary Medicine Graduate School

Poster #22

Proteomic profiling of Warmblood horses with Myofibrillar Myopathy

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Myofibrillar myopathies (MFM) in humans are caused by mutations in genes that encode sarcomere Z-disc proteins and present with progressive skeletal muscle weakness. MFM has recently been identified in Warmblood horses (WB) with exercise intolerance based on desmin immunohistochemistry and Z-disc and myofibrillar disruption in muscle biopsies. We hypothesized that specific Z-disc proteins identified through proteomic analysis would be differentially expressed in MFM WB and that their encoding genes would be candidate genes for equine MFM. The objective of this study was to compare proteomic profiles of 5 MFM WB and 4 healthy control WB. Protein was extracted from snap frozen gluteal muscle, digested in trypsin and labeled with isobaric tags (iTRAQ 10-plex) for multiplexed MS/MS quantitative analysis. Proteins were identified with the *Equus caballus* complement of UniProtKB and significance was determined by a Mann-Whitney test with *P* values adjusted for multiple testing using a Bonferroni correction. 1532 proteins were identified and 105 were differentially expressed in MFM *vs.* controls ($P < 0.00003$). Gene Ontology (GO) enrichment analysis for cellular processes identified 22 sarcomere proteins ($P = 0.0005$), 15 I-band proteins ($P = 0.026$), 13 Z-disc proteins ($P = 0.041$), and 14 oxidoreductase mitochondrial proteins ($P= 0.047$) that were overrepresented in MFM WB (FDR < 0.05). Proteins encoded by genes known to cause MFM in humans were amongst significantly upregulated proteins and represent candidate genes for further investigation of MFM in horses. With their large muscle mass and selection for athletic performance, WB horses could serve as a unique model for human MFM.

Research Grant: Mary Anne McPhail Endowment
Student Support: AVMF 2nd Opportunity Research Scholarship, MSU CMIB Graduate Group

Poster #23

Pathogenicity of emerging influenza D virus and swine influenza A virus co-infection in domestic pigs

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Influenza A viruses (IAV) are widely recognized respiratory pathogens in swine, causing fever, respiratory distress and retarded weight gain. The morbidity and mortality for IAVs can be worsened by mixed viral or bacterial infections. Emerging influenza D viruses (IDVs) were first identified in sick pigs with respiratory diseases. Our recent study suggested that, within a sample population of IAV positive feral swine (n=96), 42% had antibodies to both IAV and IDV, suggesting the host-pathogen ecology of influenza viruses possibly includes frequent co-infections with IAV and IDV. In this study our goal is to determine if co-infection of IAV and IDV creates synergistic effects in the host. We infected IAV and IDV seronegative domestic swine (n=26) with 1) 10^6 TCID50 of IAV, 2) 10^6 TCID50 of IDV, 3) 10^6 TCID50 of IAV and 10^6 TCID50 of IDV, or 4) sterile PBS and monitored clinical signs, complete blood cell counts and viral shedding in nasal swabs for 5 days post inoculation (dpi). Clinical signs were minimal and consisted of lethargy in two pigs, one each from the IAV and IDV groups, observed at 24 hours post inoculation (hpi) in the IAV group and at 24, 48 and 72 hpi in the IDV group. The pigs were euthanized at 5 dpi and all upper and lower respiratory tract tissues harvested. Next, we will quantify and compare viral shedding and viral antigens in respiratory tract tissues from the pigs across groups, and the pathogenesis in these tissues will be also be scored and compared. Our data expect to provide insight as to whether co-infection of IDV and IAV increases pathogenesis in swine compared to infection of either IDV or IAV alone.

Research Grant: Mississippi State University College of Veterinary Medicine
Student Support: Mississippi State University College of Veterinary Medicine

Poster #24

The effects of cannabidiol on neuroinflammation in experimental autoimmune encephalomyelitis

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Multiple sclerosis is a neurodegenerative autoimmune disease caused by aberrant targeting of myelin sheaths by the immune system. To model this process experimental autoimmune encephalomyelitis (EAE) is induced by immunizing mice with peptides derived from myelin proteins, such as MOG_{35-55} peptide, which causes the immune system to target the myelin sheaths of the central nervous system (CNS). Previous studies from our lab have shown that the phytocannabinoid cannabidiol (CBD) is capable of reducing clinical disease and neuroinflammation in the EAE model if given orally for 5 days after the initiation of disease. In our previous work the reduction in clinical disease at day 18 correlated with a reduction in MOG_{35-55} specific IFN-γ producing CD8 T cells in the spleen on day 10. Based on this observation, we hypothesized that the reduction in clinical disease seen with CBD for 5 days after initiation of disease would be accompanied by a reduction in neuroinflammation, with specific decreases in the CD8 T cell population within the CNS. To examine neuroinflammation more closely, CD3, CD4, and CD8 stains were used to determine the relative number and types of T cells within the CNS. In addition, GFAP and DAPI were used to stain astrocytes and nuclei respectively, which allowed us to measure the area of the lesions within the parenchyma of the CNS. As expected, there was a reduction in both lesion size and number of T cells present within the CNS with CBD treatment; however, the reduction was modest, suggesting that other factors also contribute to the neuroprotective capabilities of oral CBD in the EAE model.

Research Grant: P20GM103646
Student Funding: MSU-CVM

Poster #25

Biochemical effects of oral chlorpyrifos in lungs of neonatal and adult mice

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Chlorpyrifos (CPF) is an organophosphate (OP) pesticide known to exhibit toxicity via inhibition of acetylcholinesterase (AChE) in the nervous system. We previously showed that endocannabinoid (eCB) metabolizing enzymes were even more sensitive to inhibition by OP pesticides than AChE in neonatal rats, leading to increased levels of eCBs in brain. Because eCBs are known to have immunosuppressive effects, we are investigating a link between eCB metabolism and immunity in adults and neonates exposed to CPF. We hypothesized that neonatal mice would be more sensitive than adult mice to the effects of CPF on eCB metabolism. Adult mice (≥ 8 weeks old) and neonatal mice (post-natal day 10) were treated with CPF (2.5 mg/kg oral) or vehicle daily for 7 days. Tissues were harvested 4 hr after final treatment. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) hydrolysis activities in spleen and brain were not different between vehicle and CPF, but lung 2-AG hydrolytic activity was decreased by CPF in adults. Lung microsomes from both age groups demonstrated marked inhibition of carboxylesterase (Ces) activity, one of the known eCB metabolizing enzymes. Lung Ces activity in neonates was more sensitive to CPF than adults. Activity-based protein-profiling (ABPP) and immunoblotting of lung microsomes confirmed that Ces1 was present in both age groups, and the activity was inhibited by CPF. ABPP-mass spectrometry of neonatal mouse lung microsomes identified 31 serine hydrolases, and Ces1d (the murine orthologue of human CES1, abundant in human macrophages) was selectively inactivated by CPF. Further studies in both age groups will explore the role of inhibition of Ces1d by CPF in pulmonary inflammation.

Funding: Mississippi State University College of Veterinary Medicine
Student Support: MSU-CVM and NIH Grant R15GM128206

Poster #26

Comparative analysis of TRIM9 expression in phagocytes

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The persistence of an immune response can contribute to the development of disease states, increasing the extent of tissue damage or perpetuating the disease condition. Chronic inflammation is often characterized by high numbers of macrophages and neutrophils that release ROS, cytotoxic factors, and cytokines, further amplifying inflammation. Phagocytes have been shown to contribute to disease in both laboratory-induced and natural models of chronic inflammation in veterinary species. Inflammatory bowel disease, ischemic injury and allergies are a few examples of inflammatory conditions shared by dogs, horses, and humans. Studying the cellular mechanisms required for phagocyte migration to sites of inflammation will elucidate how these cells could be therapeutically targeted during persistent inflammation. The E3 ubiquitin ligase TRIM9 is highly expressed in the brain and mediates axon migration and synaptic vesicle release. Our lab first reported TRIM9 expression in zebrafish phagocytes and demonstrated that disrupting its function in vivo alters macrophage cell shape and motility. We hypothesize that TRIM9 expression is regulated by immune stimulation and plays an essential role in regulating mammalian phagocyte function. For this work, we will characterize TRIM9 expression in dog, horse, mouse and human phagocytes. Furthermore, we aim to determine TRIM9's role in immune functions, such as migration and phagocytosis and begin to define the TRIM9 protein interactome. This comparative analysis of the molecular mechanisms involved in phagocyte migration and function has great potential for producing targeted therapeutics for both human and veterinary patients that suffer from persistent damaging inflammatory states.

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Student support: none

Poster #27

Biological sex influences mast cell activation patterns and associated disease severity

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Highly prevalent and debilitating mast cell (MC)-associated diseases, including allergy, irritable bowel syndrome, and autoimmune disorders exhibit a sex bias with females at greater risk. The mechanisms underlying these sex differences remain poorly understood. Our previous studies utilizing MC-dependent models of IgE-mediated anaphylaxis and psychological stress, demonstrated that female mice exhibited more severe pathophysiology and increased MC mediator release compared to males. The objective of this study was to understand the mechanisms of sexual dimorphism in MC disease. Our hypothesis is that heightened disease in females is due to sex-specific differences in MC activation patterns. To investigate, we performed transcriptional analysis on IgE-DNP allergen-activated male and female bone marrow-derived MCs (BMMCs). Using Ingenuity software, we discovered a similar pattern of IgE-DNP activated pathways between male and female BMMCs. However, many of these pathways were upregulated to a greater extent in female BMMCs including sphingosine-1-phosphate signaling, leukocyte extravasation, NF-κB signaling, and chemokine signaling. Our analyses also revealed up- and downregulation of sex-specific pathways. For example, male BMMCs exhibited upregulated pathways of transcriptional repression and downregulated pathways of cell cycle control while female BMMCs exclusively exhibited upregulation of histidine degradation and downregulation of amino acid biosynthesis. Together, these studies reveal new insight into sex differences in MC biology and disease that correlate with the female sex bias in humans. Further elucidating the role of sex in MC activation is likely to provide novel therapeutic approaches to MC diseases.

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Poster #28

Adaptation of tilapia (*Oreochromis niloticus*) pituitary culture to an intermittent flow respirometry system reveals endocrine and environmental regulation of oxygen consumption rate

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An organism's ability to regulate it's metabolic rate in the face of physiological and environmental stressors is essential to survival. Thusly, the ability to measure oxygen consumption in an organism, organ, or tissue is of particular interest to researchers from a wide variety of fields. Intermittent flow respirometry (IFR) is a powerful experimental procedure typically used to measure oxygen consumption rate (OCR) in aquatic organisms. In this technique, short periods of closed-chamber oxygen consumption measurements are interspersed with regular flushing. This method allows for the regular measurement of OCRs over long periods of time without total depletion of oxygen or the build-up of waste. For these reasons, IFR has become an important part of the aquatic physiology toolbox. However, to our knowledge, no one has used IFR to measure oxygen consumption in cultured tissue or organs. In the present study, the culture of the rostral pars distalis (RPD) of the tilapia (*Oreochromis niloticus*) pituitary gland was adapted to an intermittent flow respirometry system. Tilapia are phenomenal osmoregulators, able to acutely withstand wide fluctuations in environmental salinity. This physiological response depends on the actions of the RPD hormone prolactin. Thus, the metabolic rate of the RPD is high, but tunable. Once the culture was validated, the intermittent flow respirometry system was used to probe the effects of osmolality and the catabolic stress hormone leptin on pituitary OCR. Hyper- and hypoosmotic culture media were associated with decreased and increased OCRs, respectively. Additionally contrary to what is seen in mammals, our results suggest leptin acts to decrease the OCR in the tilapia pituitary.

Research Grant: NSF
Student Support: GAANN Fellowship

Poster #29

Knock-down of *TMEM150A* results in increased cytokine production

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Transmembrane Protein 150A (TMEM150A/TM6P1/DRAM5) is a member of the TMEM150/damage-regulated autophagy modulator (DRAM) family of proteins that possess six-transmembrane domains with both termini positioned within the cytoplasm. Five members of this family have been reported in human, and of those, three have been implicated in regulating autophagy. Although autophagy is most well-known for cellular homeostatic degradation of organelles and cell survival processes, autophagy has also been linked to immune-related functions. For example, the engagement of Toll-like receptor 4 (TLR4) by the lipopolysaccharide (LPS) component of gram negative bacteria can induce autophagy. Based on the observations that multiple TMEM150/DRAM family members have been implicated in autophagy and that, through its interactions with phosphatidylinositol 4-kinase type III alpha (PI4KIIIa), TMEM150A can regulate the production of PI(4,5)P2 (a component in the TLR4 pathway), we asked if there is a functional connection between TMEM150A and TLR4 signaling. Knock-down of TMEM150A in cell culture led to significant increases in TLR4-induced cytokine secretion as well as increases in cytokine transcript levels. Parallel experiments demonstrated that knock-down of TMEM150A triggered dramatic alterations in the activity of multiple transcription factors suggesting that TMEM150A functions to regulate immune-related gene expression. In summary, we provide the first evidence that TMEM150A plays a role in regulating cytokine production likely through a global role in regulating transcription factor activity.

Poster #30

Characterizing a Naturally Occurring Canine Model of Neuropathic Pain

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Chiari-like Malformation and Syringomyelia (CMSM) is a highly prevalent disease complex in Cavalier King Charles Spaniels (CKCS) that affects the brain and spinal cord and causes severe neuropathic pain and itch. This condition is widespread and naturally-occurring in CKCS making it an ideal translational model of Chiari-type 1 malformation (CM1), a similar condition in humans, and for studying neuropathic pain. The biggest challenge in any study of pain is reliable quantification. Therefore, the purpose of this study was to obtain measureable data on pain in a cohort of CKCS and to compare these data with the presence and severity of CMSM determined by MRI. Fifty-two client-owned dogs were recruited and owners were asked to complete two questionnaires. Dogs underwent neurological examinations and MRI. Specialized hemostatic forceps were used for mechanical quantitative sensory testing (QST) on the neck. Thirty-four dogs had SM and 36 were painful on neurological examination. Owners reported scratching in 35 dogs, pain in 30, and 28 were reported to have both pain and scratching signs. There was no significant difference between the presence or severity of SM and pain on neurological examination, QST data, or owner-reported data ($P > 0.05$). However, dogs that were painful on neurological examination had significantly lower thresholds to mechanical testing with a mean threshold of 2.1kgs (SD: 0.76kg) compared to those that were not painful (mean = 2.7 kgs, SD = 0.54kg) ($P = 0.003$). These findings show that mechanical QST may be a useful tool for bridging the gap between at home and in hospital observations as well as for clinical trials. Also, the relationship between CMSM and pain deserves deeper examination.

Research Grant: The American Cavalier King Charles Spaniel Club Charitable Trust through the American Kennel Club Canine Health Foundation
Student Support: George H Hitchings New Investigator Award; AVMA/AVMF 2nd Opportunity Summer Research Award

Poster #31

Antimicrobial resistance dynamics in foodborne *Campylobacter coli* isolates using Markov random field networks

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Antimicrobial resistance threatens public health by reducing the efficacy of antimicrobial treatments for infection. Use of antimicrobial drugs may select for resistance to those drugs as well as other drugs in unrelated classes. Consequently, collateral resistance, which may result from various mechanisms of genetic linkage in bacterial genomes, may drive emergence and persistence of multidrug-resistance (MDR). Traditional analytical methods focus on resistance to individual drugs, but are less suitable for evaluating MDR dynamics because they do not quantify joint distributions of minimum inhibitory concentration (MIC) for multiple drugs. This study evaluated phenotypic MDR dynamics using Markov random field networks (called "R-nets"). We hypothesized that collateral resistance influences MDR evolution, as reflected through correlated phenotypic MICs of unrelated drugs after controlling for confounding variables. To test this hypothesis, we used R-nets to quantify joint MIC distributions to nine antimicrobial drugs among *Campylobacter coli* isolated from agricultural swine herds experiencing varying degrees of antimicrobial exposure. We found high levels of macrolide- and tetracycline-resistance in *C. coli* isolates from pigs that were and were not exposed to antimicrobials. Using R-nets, we found that MICs for macrolides and tetracycline were associated with one another, and with MIC for unrelated drugs like ciprofloxacin. These results provide phenotypic support for the plausibility of collateral resistance in these populations of *C. coli*. Future studies will use these same isolates to identify genetic linkages of macrolide-, tetracycline-, and ciprofloxacin-resistance, given their importance to clinical medicine.

Research Grant: None

Student Support: NCSU Provost's Fellowship

Poster #32

Cutaneous *Malassezia* spp. dysbiosis in canine allergic dermatitis by next-generation sequencing (NGS)

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DNA sequencing technologies have revolutionized the study of skin commensals, how they contribute to health, and their role in disease. The purpose of this study is to classify skin-associated *Malassezia* to the species level with phylogenetic analysis of NGS data, and to confirm with real-time quantitative PCR (qPCR). DNA previously extracted from skin swabs (n=192) of 10 healthy dogs, 8 non-lesional allergic dogs, and 8 laboratory dogs with induced atopic lesions were included in this study. *Malassezia* NGS sequences from all 192 samples were speciated by alignment to a published *Malassezia* spp. database with the open-source bioinformatics software Pplacer. Quantitative PCR targeting *M. pachydermatis*, *M. restricta* and *M. globosa* confirmed that *M. pachydermatis* was significantly more abundant on swabs taken from the laboratory colony of dogs compared to client owned healthy and allergic dogs ($P<0.05$). Phylogenetic analysis of 37,000 NGS *Malassezia* sequences from healthy and non-lesional allergic dogs showed *M. restricta* was more abundant on healthy skin ($P<0.05$), while *M. pachydermatis* was more abundant on non-lesional allergic skin ($P<0.05$). Molecular methodologies are proving to be useful in the study of skin commensals, and may indicate that *M. restricta* and *M. globosa* act as protective commensals on healthy skin, while the pathogenic *M. pachydermatis* dominates the skin of non-lesional allergic dogs. Comparisons of quantitative and relative abundance methods are not always consistent and should be considered in the choice of methods for future studies.

Research grant: self-funded.
Student support: self-funded.

Poster #33

Contributing effect of organochlorine exposure on Atlantic bottlenose dolphin deaths from morbillivirus

Jennifer Cossaboon, Birgit Puschner, Gina Ylitalo, Deborah Fauquier, and Tracey Goldstein

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Dolphin morbillivirus (DMV) is a substantial threat to cetacean populations due to its immune suppression effects, interspecies transmission, and high mortality rate. Though a direct causal link has not been made, chemical pollution has been implicated in potentially increasing susceptibility to DMV infection in nearshore cetaceans. A recent outbreak along the US East Coast resulted in the death of over 1600 Atlantic common bottlenose dolphins. We hypothesize that dolphins that died from morbillivirus infection had significantly higher mean ΣPCB and mean ΣDDT concentrations in blubber and brain tissue compared to bottlenose dolphins that inhabited the same areas and stranded before the DMV outbreak. Paired brain and blubber samples from 15 confirmed DMV cases and ten control dolphins are being analyzed by GC/MS for 51 PCB congeners and six DDT-related compounds. Contaminant levels in brain tissue had not been measured previously in DMV cases despite the brain being one of the primary locations for pathological lesions. Preliminary results from this study indicate that there are substantially higher PCB concentrations in the brains of DMV cases than previously reported in dolphins that did not die from the virus. Lipid content and lipid classes are also being determined and will reveal how individual congeners partition between blubber and brain tissue in dolphins that died from DMV versus the control dolphins that died from other causes. Additionally, a new organic contaminant extraction procedure is being evaluated on a subset of the samples using Phree phospholipid removal plates that may provide a more efficient, high-throughput methodology for future biomonitoring of contaminants in marine mammal tissues.

Research Grant: National Marine Fisheries Service

Student Support: “Students Training in Advanced Research” Fellowship (School of Veterinary Medicine endowment funds, Goldstein Lab funding)

Poster #34

Evaluation of a novel soluble epoxide hydrolase inhibitor in the UC Davis Type 2 Diabetes Mellitus Rat Model

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Type 2 Diabetes Mellitus (T2DM) is a serious and increasingly prevalent metabolic disease that is characterized by increased blood glucose levels, insulin insensitivity, and ß-cell/islet dysfunction. One of the central components of the pathophysiology of T2DM in humans is a general inflammatory state which can be prophylactically or therapeutically targeted. One potential way of targeting this dysregulation is via soluble epoxide hydrolase inhibitors (sEHI) which have been shown to have anti-inflammatory effects. As a part of Dr. Peter Havel's laboratory and in collaboration with Dr. Bruce Hammock's laboratory we investigated the therapeutic properties of a novel sEHI in the UC Davis Type 2 Diabetes Mellitus rat model. During this pilot study we are performed a dual treatment of fish oil and the sEHI on the UC Davis T2DM Rat Model, targeting the inflammatory etiology. The treatment and control groups were n=6. The goal of the study is to see if sEHIs have a protective effect and delay T2DM development in the UC Davis T2DM rat model. From our preliminary results we saw some positive effects from the fish oil and sEHI on the treatment group as shown by: delayed diabetes onset; lower blood glucose, triglyeride, and total cholesterol levels; and improved oral glucose tolerance test results. These preliminary results suggest sEHIs may have a positive effect by delaying onset of T2DM and that further investigation is warranted.

Research Grant: Dr. Havel's Discretionary Funding
Student Support: Students Training in Advanced Research (STAR) Program

Poster #35

L. plantarum repairs intestinal epithelial barrier damage in chronic SIV infection through *PPARa* mediated-mitochondrial rescue

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HIV infection causes persistent inflammation and 'leaky gut' as a result of immune dysfunction and epithelial barrier disruption. Despite effective anti-retroviral therapy (ART), complete immune recovery and gut barrier integrity are not achieved. Novel approaches to restore the gut microenvironment are imperative to position the immune system for viral eradication. To identify mechanisms for intervention, we performed intestinal loop surgeries using the nonhuman primate model of AIDS to capture resident intestinal microbiota, tissue microenvironments, and their interactions. Probiotic *L. plantarum* was injected into ileal loops to identify host and bacterial contributions to mucosal responses in SIV infection. We found that disruption of epithelial tight junctions in chronic SIV infection was repaired after 5-hour exposure to *L. plantarum* independent of CD4+ T cell recovery. Metabolomic and gene expression analyses revealed that mitochondrial dysfunction and impaired beta-oxidation were associated with SIV infection and reversed after exposure to *L. plantarum.* Activation of *PPARa* signaling by *L. plantarum* restored mitochondrial activity in chronic SIV infection. Our findings in Caco-2 cell cultures suggest that epithelial barrier function is regulated by ATP-linked respiration, which is attenuated during SIV/HIV infection and can be augmented by *PPARa* modulation. Integration of HIV-induced mitochondrial dysfunction with microbiota-mediated changes in the gut provides new strategies for HIV disease management and optimization of tissue recovery during ART.

Research Grant: **NIH R01 AI123105**

Student Support: NIH F30 GM131457, UCD SVM

Poster #36

Decoding ERK Regulation of the Cell Cycle to Direct Rational Combinatorial Therapy

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One hallmark of cancer is uncontrolled cell proliferation, and cancer cells often carry mutations in the Ras/MAPK pathway that regulate cell cycle events. ERK, the terminal kinase of the MAPK pathway, increases the expression of cyclins, which control the cell cycle in complex with cyclin-dependent kinases (CDKs). Paradoxically, while ERK is necessary for cell proliferation, excessive activity can induce cell senescence. ERK is an important pharmacologic target for anticancer therapies; however, durable and potent ERK suppression is clinically difficult to achieve. This study investigates the combination of ERK and CDK inhibition for synergistic suppression of proliferation. Using live-cell microscopy and improved fluorescent reporters, ERK activity was quantified throughout the cell cycle at a temporal resolution that was previously inaccessible. Regulation of cyclin-dependent kinase inhibitor proteins (CKIs) will be examined to determine the mechanistic basis for changes in proliferative threshold. Together, this work will provide the data needed to optimize combined CDK and ERK inhibition for reduced tumor growth.

Funding: Boehringer-Ingelheim Animal Health (BI); NIH R01

Poster #37

ELISA Competition Assays for Antibodies Capable of Blocking PD-L1Ig

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PD-1 is an inhibitory receptor that binds to PD-L1 on the surface of tumor and antigen presenting cells in the tumor microenvironment, causing suppression of T-cell activity and tumor progression. Human clinical trials with anti PD-1 monoclonal antibodies, nivolumab and pembrolizumab, showed an objective response rate (ORR) of 30% to 40% with melanoma and 87% with relapsed or refractory Hodgkin's lymphoma. We have previously explored the development of canine PD-1/PD-L1 reagents for potential veterinary applications. 5 antibodies capable of blocking PD-1Ig and PD-L1Ig were previously developed. In this study, we are further investigating the nature of epitope binding by four antibodies by performing ELISA competition assays. These canine reagents have multiple diagnostic and therapeutic applications in the context of canine oncology.

Poster #38

SYCP3 Impairs BRCA2 Increasing Risk of Cancer

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BRCA2 maintains genomic integrity by functioning in homologous recombination (**HR**) and thereby suppresses tumor formation. BRCA2 recruits other proteins such as RAD51 in somatic cells and RAD51 and DMC1 in germline cells to function in key steps of HR. Loss of BRCA2 function is associated with increased risk of breast and other cancers. Mechanisms that inactivate BRCA2 function other than direct mutational inactivation are yet to be determined. My research addresses this gap in knowledge and defines a new mechanism by which misexpression of the germline protein SYCP3 in somatic cells inhibits BRCA2-mediated HR.

SYCP3 is an essential structural component of the meiosis-specific synaptonemal complex. SYCP3 is typically expressed only in germline cells but not in somatic cells. Emerging evidence indicates that SYCP3 is misexpressed in certain cancers. The structural role of SYCP3 in meiosis is well understood but not much is known about its potential effects in somatic cells. Recently, it was reported that in somatic cells SYCP3 interacts with BRCA2 and impairs recruitment of RAD51 involving mechanisms that remain to be defined. The goal of my research is to establish the mechanism, by which SCYP3 regulates BRCA2 function in HR**.** My hypothesis is that SYCP3 regulates the differential interaction of BRCA2 with RAD51 and DMC1.

Specific Aim 1 will establish the biochemical mechanism by which SYCP3 regulates BRCA2 function by *in vitro* assays using purified proteins. Specific Aim 2 will use cell-based models to determine the biological significance of the interaction between SYCP3 and BRCA2 on HR efficiency.

The findings from this proposal will establish SYCP3 expression in tumors as a potential biomarker for HR deficiency.

Research Grant: None
Student Support: MCB NIH T32 (T32 GM007377)

Poster #39

Viral RNA titers in kidneys collected from birds experimentally infected with infectious bronchitis virus

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Infectious bronchitis virus (IBV), a highly contagious viral disease of chickens with global distribution, is of primary concern in the poultry industry due to economic losses incurred from affected birds. The virus initially infects the upper respiratory tract, resulting in deciliation of the mucociliary apparatus. IBV can also replicate and destroy other epithelial surfaces. In layers, lesions in the oviduct lead to declines in egg production and quality, while in broilers economic losses are primarily due to poor weight gain and feed efficiency. In addition, the IBV-QX strain is nephropathogenic, causing severe nephritis and high mortality. Current understanding of IBV-QX pathogenicity is limited. Thus, the objectives of this study were to determine if there are differences in kidney IBV viral titers in 1) layer versus broiler chickens, and 2) chickens inoculated with wild-type IBV-QX compared to the derivative QX vaccine. In total, 58 specific pathogen free (SPF) layers and 58 SPF broilers were included in this study. Of these, 24 of each were inoculated intranasally with the vaccine strain, 24 of each with the wild-type strain, and 8 of each were mock-inoculated as a control. Chickens were euthanized serially from day 1 through 8 post-inoculation, and kidney viral titers were determined using RT-qPCR. No significant differences were found between layers and broilers, however chickens inoculated with the wild-type virus had significantly higher titers than those inoculated with the vaccine strain. This information will be combined with histological and immunohistochemistry data from the same study to elucidate whether the presence of lesions and viral protein expression corresponds with viral RNA presence.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Poster #40

Mump Virus Nucleoprotein Domain "Top" Affects Defective Interfering Particle Production.

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Mumps virus (MuV) is a negative-sense single-stranded RNA virus belonging to the family Paramyxoviridae. A human pathogen, MuV is responsible for acute infection of the parotid glands, and can cause severe cases of encephalitis, meningitis, and deafness. The nonsegmented RNA genome of MuV is encapsidated by the nucleocapsid protein (NP), which forms the ribonucleoprotein (RNP) complex – which serves as a template for RNA synthesis. To make RNA accessible to the viral polymerase, a conformational change within NP must occur. Crystal structure analysis of the NP of parainfluenza virus 5 (PIV5), a paramyxovirus closely related to MuV, indicates that an α-helix close to the RNA genome becomes flexible when RNA is removed. This region of the NP is likely responsible for the conformation change which allows the polymerase to access RNA for transcription and replication. To examine the functionality of MuV's NP, point mutations were made in MuV NP protein corresponding to PIV5 at sites G185P, A197Q, Q200R, and groups denoted as Top (N63G, P139D, A197Q), Tip (P109E, N121G, A124R), and Bottom (G21S, S29T, P43N, R93Q, R304Q). The "Top" MuV mutant exhibited normal growth kinetics at low multiplicity of infections (MOIs); however, at high MOI's the virus could not efficiently replicate. Further analysis indicates that production of defective interfering (DI) particles was enhanced in the mutant virus. Understanding the production of DI particles, which can lead to increased interferon production, will invariably lead to a better understanding of MuV pathogenesis as well as its replication/ transcription process.

Research Grant: National Institute of Health Grant RO1AI106307

Student Support: None

Poster #41

Cytokine expression following porcine islet xenografts in immunosuppressed and tolerant nonhuman primates

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Pancreatic islet cell transplantation has demonstrated value as a therapeutic modality in Type 1 diabetes that can restore insulin independence and lower risk of secondary complications. Although highly effective, scarce human donor pancreata has limited its widespread use. One potential solution is the use of porcine islets, which produce a near-identical insulin. Porcine islet xenografts undergo rejection via a primarily T-cell-mediated process in nonhuman primate (NHP) models. Xenograft survival depends on mitigating this response, via either long-term immunosuppression, or ideally, inducing a state of immune tolerance to the graft. Cytokines help regulate the balance between promotion and impairment of graft survival, some enforcing tolerance by inhibiting immune cells, and others breaching tolerance by causing cytotoxic damage. Characterizing cytokine profiles in xenotransplanted NHPs exposed to either conventional T-cell directed immunosuppression or tolerogenic protocols has the potential to uncover the molecular basis of regulatory mechanisms that foster tolerance and prolong graft survival. We studied the systemic cytokine response to islet xenotransplantation in 17 diabetic cynomolgus macaques that received either immunosuppression or a tolerogenic protocol. Cytokines were measured with a multiplex assay from serum samples collected at serial time points. We analyzed cytokine expression over time, and compared these patterns across NHPs stratified by graft functional outcomes. In the future, we plan to study how cytokine expression varies by compartment, to evaluate accuracy of cytokine profiles in predicting the fate of transplanted islets, and to exploit tolerogenic mechanisms with targeted cell therapies.

Research Grant: National Institutes of Health Grant U01AI120130
Student Support: Boehringer Ingelheim Veterinary Scholars grant

Poster #42

RNA-Seq identifies mutually exclusive driver insertions in PI3K-hyperactive mammary carcinomas

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Abstract: The most common carcinomas in women, and one of the leading causes of cancer-related death, are invasive mammary tumors. Creating *in vivo* models that accurately recapitulate mammary tumorigenesis is difficult due to high genetic heterogeneity within and between tumors. Activating mutations in the catalytic subunit of PI3K are one of the most common genetic alterations in human breast carcinomas. We conducted a forward genetic screen using a Cre-inducible *Sleeping Beauty* (SB) transposon system in the context of PI3K hyperactivation to discover genetic events that cooperate with an oncogenic PI3K mutation. The T2/Onc3 transposon activates endogenous proto-oncogenes or inactivates tumor suppressor genes by insertional mutagenesis. In conjunction with a R26-LSL-SB11 transposase and tissue-specific Cre, the transposon mobilizes and integrates into the genome. Mice with the PI3K mutation and SB had accelerated tumorigenesis compared to controls. RNA sequencing showed molecular subtypes with correlating T2/Onc3-induced insertion mutations, including estrogen receptor (ER) positive and negative subtypes. RNA and DNA sequencing defined common insertion sites of the SB transposon, allowing for detection of frequently mutated genes. We analyzed 244 tumors and identified 20 tumor suppressor genes and 35 oncogenes as common insertion sites. Notably, some common RNA insertion sites (R-CIS) were mutually exclusive, specifically insertions in *Jup*, *Hras*, *Pten*, and *Notch1*. Additionally, *Pten* insertions were associated with ER+ tumors. This model provides a source of genetically heterogenous mammary tumors with the same initiating mutation (PI3K) useful for identifying cooperating pathways and drivers of specific tumor phenotypes.

Research Grants: American Cancer Society Research Professor Award (to D.A.L.), Genentech/Roche Inc.
Student Support: Boehringer Ingelheim Veterinary Scholars Grant

Poster #43

SOS1 Expression in Canine Leukemia

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Oncogenes are genes that have the potential to cause cancer and are often mutated or highly expressed in tumor cells. The majority of the 'more easily' discovered oncogenes have been identified and such information has frequently been used to develop effective therapies. However, approaches used to identify oncogenes typically fail to detect copy number neutral genomic rearrangements, such as microinversions. These later genomic rearrangements may represent a group of mutations that play a substantial, albeit unappreciated, role in cancer.

B cell acute lymphoblastic leukemia (B-ALL) is the most common form of childhood cancer. And malignant lymphoproliferative diseases also account for approximately 40% of canine neoplasia. *Sos1* is frequently mutated in B-ALL and its expression corresponds with decreased survival in previous human and mouse studies. We explored whether a novel intrachromosomal microinversion in canines creates a similar truncated form of SOS1 in leukemias. Inhibition of SOS1 protein could result in patient remission and elimination of affected B cells. This study will investigate a novel change in SOS1 that may be present in leukemia and other types of cancer and thus be a potential therapeutic target using 5'-/3'-RACE and qPCR. Results showed only a minimal increase in the expression of the truncated Sos1 in canine leukemia samples but a dramatic increase in expression in canine osteosarcoma samples.

Research Grant: Unknown
Student Support: University of Minnesota Summer Scholars Program

Poster #44

Gender Dependent Alterations in the Mechanical Response of Collagen V Haploinsufficient Murine Tendons

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Gender-specific differences in body structure and composition may exacerbate the detrimental changes present in pathological tendons in Classic Ehlers-Danlos syndrome (EDS) patients. We hypothesized that female *classic* EDS mice will have inferior tendon mechanical properties compared to male *classic* EDS mice, but there will only be differences in structural properties due to gender in wild type tendons.

Adult male and female uninjured WT C57/BL6 and HET EDS mouse patellar tendons were used. Compared to WT females, WT male tendons had significantly higher failure load, failure stress, tissue modulus and tissue stiffness. Additionally, WT males exhibited significantly elevated dynamic modulus at 10Hz and across all strains (2%, 3%, 4%). HET male tendons had a significantly higher failure load than HET females, with no difference observed in tissue stiffness. HET male and female mice showed trending differences at 2% strain, 0.1 and 1 Hz, and at 4% strain, 1 Hz. Male HET tendons trended towards a decrease in stiffness compared to WT tendons, with no other difference between genotypes.

WT male patellar tendons demonstrate superior material and structural properties compared to WT female tendons. Conversely, the same differences in structural properties seen in WT tendons were not present in HET mice. This contrasting finding indicates that the structural properties of HET male tendons were affected by the reduction of type V collagen to a greater degree than the structural properties of HET female tendons, after considering the inherent gender-differences. This study demonstrates that sex plays a tendon-specific role in tendon health, and can influence the degree to which tendon properties of *classic* EDS mice are affected.

Research Grants: This study was supported by AR065995, AR044745 and the Penn Center for Musculoskeletal Disorders (P30 AR069619).
Student Support: None.

Poster #45

The genome-wide DNA-binding properties of the ALS-associated protein TDP-43

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TAR DNA-binding protein 43 (TDP-43) is the major protein component of neuronal inclusions characterizing the fatal neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-TDP). Disease-causing mutations in the gene that encodes TDP-43, *TARDBP*, further implicate TDP-43 in disease pathogenesis. Originally identified as a protein capable of binding HIV trans-activation response element DNA, research has emphasized TDP-43's role as an RNA-binding protein, thereby neglecting a fundamental property of TDP-43 biology. To address this, we performed chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) in human cells, demonstrating that TDP-43 is a genome-wide DNA binding protein. Notably, TDP-43 was particularly enriched at small nuclear RNA genes and ALS-causative mutations in *TARDBP* impaired TDP-43's ability to bind these loci. These findings point to a potential role for TDP-43 in snRNA biogenesis that may contribute to the disrupted splicing profiles characteristic of ALS and FTLD-TDP.

Research Grants: National Institutes of Health (NIA K08 AG033101 to ACP), the Burroughs Wellcome Fund Career Award for Medical Scientists (to ACP) and the Benaroya Fund (to ACP)
Student Support: Mentor (ACP), University of Pennsylvania Biomedical Graduate Studies

Poster #46

Implications of free-ranging dog movement patterns for urban rabies control

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In 2015, a case of canine rabies in Arequipa, Peru indicated the re-emergence of rabies in the city. Despite vaccination campaigns and ring euthanasia of free-ranging dogs around positive cases, the outbreak has spread throughout the city resulting in new cases every week. The aim of this study was to explore how the urban landscape of Arequipa affects the spread of canine rabies and corresponding control measures by examining movement patterns of free-ranging dogs. To do this, we tracked 23 free-ranging dogs using Global Positioning System (GPS) collars. We analyzed the spatio-temporal GPS data using the time- local convex hull method; around every recorded location, we constructed convex polygons (hulls) using a selected set of nearest neighbor locations. We used these hulls to approximate space usage by the dogs, including home range and utilization area, as well as, directionality of movements, duration of stay in different areas, and revisitation to different areas. We found that there was great variation in movement patterns between individual dogs. The movement patterns were affected by local environments. Specifically, water channels, an urban feature of Arequipa that are dry most of the year, were found to be areas of high movement. Dogs that utilized the water channels had distinct movement patterns. These findings suggest that some dogs using the water channels as 'highways' have the potential to spread disease far beyond the ring euthanasia control practices. As these practices have led to local resentment, control efforts should focus on a robust vaccination campaign not limited to areas where there have been recent cases.

Research Grant: Departmental Funds - Department of Biostatistics, Epidemiology & Informatics, Perelman School of Medicine, University of Pennsylvania.
Student Support: Medical Scientist Training Program Grant

Poster #47

The SOS response is a Critical to Bacterial Colonization of the Mammalian Gut

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Background: The ability of bacteria to rapidly adapt to environmental challenges has profound implications for bacterial infections. Bacterial stress response pathways enable bacteria to surive environmental challenges. The SOS response is a stress response pathway that activates in response to DNA damage and promotes DNA repair. The SOS response is vital for bacterial survival through its repair of DNA, but significantly, culture-based experiments describe the SOS response as being a major contributer to adaptive mutagenesis and horizontal gene transfer, both of which increase genomic plasticity ultimately enhancing survival, virulence, and adaptation. In addition to modifying the genome, *in vitro,* the SOS response controls some virulence factors, which may be critical for bacterial colonization and pathogenesis. Despite a large body of research describing the important consequences of the SOS response, little work has been done to interrogate the particular host environments that induce and require the SOS response for survival.
Results: Using a mouse commensal *E. coli* strain, I demonstrate that the SOS response is critical for colonization of the murine gut both in the presence and absence of gut inflammation. In a healthy gut environment a SOS deficient strain is impaired for colonization relative to a wildtype strain. Further, in the presence of acute gut inflammation the SOS deficient strain maintains this fitness defect.
Significance: My results highlight the importance of the SOS response for *E. coli* in the mammalian gut. Understanding the molecular mechanisms governing bacterial adaptation and virulence provides a new paradigm for combating bacterial infections that aim to impede pathogenesis and evolution.

Research Grant: Burroughs Wellcome Trust Fund
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Poster #48

A genetic-based vaccine overcomes maternal antibody inhibition of immune responses

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Infants are particularly vulnerable to infections and severe disease, including from influenza virus. One increasingly promising strategy to protect them during this period is through maternally derived immunity transferred to the neonate. Maternal antibodies (matAb) can protect the infant soon after transfer but wane over time, leaving the infant vulnerable again. Therefore, active immunity via vaccination must also be generated in the infant. Here, using a mouse model we show that influenza virus-specific matAb inhibit the development of infant antibody responses after infection with live influenza virus or vaccination with inactivated virus. However, a novel mRNA-based vaccine expressing an influenza virus protein was able to generate strong antibody responses in mice that possessed influenza virus-specific matAb, leading to long-lasting protection. This vaccine overcomes matAb inhibition through long-term antigen expression and sustained germinal center activity and does not require cell-surface antigen expression. Together, these results suggest that genetic vaccines can overcome matAb inhibition and elicit potent immune responses in infants.

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Poster #49

Transplantation of human pluripotent stem cell-derived optic vesicles in a rat model of retinal degeneration

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Photoreceptor (PR) death is a leading cause of blindness worldwide, and few treatment options currently exist for affected patients. However, human pluripotent stem cell (hPSC)-derived retinal organoids have recently emerged as a source of photoreceptors for developing cell-based therapies for blinding retinal diseases. Our work is therefore focused on evaluating stem cell-based PR replacement therapy in the Foxn1-S334ter rat, a model of retinal degeneration. Using a hPSC-CRX+/tdTomato reporter line and the Gamm lab's previously described protocol for differentiation, retinal organoids were generated and transplanted into the subretinal space of adult rats with degenerate retinas. Eyes from transplanted rats were assessed at 1, 3, and 6 months post-transplant for rod and cone differentiation, host bipolar cell dendritic outgrowth, and synapse formation. Anatomic evidence of integration including host bipolar cell sprouting and synaptic protein expression was present as early as 2 weeks post-transplant. By 6 months, hPSC-derived photoreceptors dominated the hPSC-derived donor cell population in the Foxn1-S334ter rat subretinal space, many of which expressed mature rod and cone markers, and proliferative retinal progenitor cells were at a minimum. As such, transplantation of hPSC-derived retinal organoids has the potential to reconstruct the photoreceptor layer following severe host photoreceptor degeneration and further studies of transplant safety and efficacy in rodent models are warranted.

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Poster #50

Analysis of biotransformation of environmental bladder carcinogens by canine glutathione S-transferases

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Transitional cell carcinoma of the bladder (TCC) has been associated with certain environmental chemical exposures in both humans and dogs. Glutathione S-transferases (GSTs) are important biotransformation enzymes for reactive environmental chemicals, and polymorphisms affecting the function of GSTs from the mu-, pi-, and theta-classes have been associated with TCC risk in humans. Dogs also have polymorphic GST mu (GSTM1) and pi (GSTP1) isoforms, and two polymorphic GST theta isoforms (GSTT1 and GSTT5). However, it is not known which canine GSTs are important for detoxification of environmental chemicals that have been associated with TCC risk. The aim of this study is to identify which, if any, GST isoforms in dogs catalyze the biotransformation of compounds from five categories of candidate environmental bladder carcinogens, including acrolein, 4-aminobiphenyl, sodium arsenite, 4-chlorophenol, and bromodichloromethane. We hypothesize that one or more canine GST isoforms detoxify these environmental bladder carcinogens. A newly validated, generalizable HPLC method for detecting glutathione depletion will be used to measure *in vitro* glutathione-conjugation activity of cloned canine GSTM1, GSTP1, GSTT1, and GSTT5 enzymes towards each compound. GST isoforms with the highest activities for each compound will be tested for effects on substrate mutagenicity compared to the parent compound using a micronucleus assay for DNA damage in canine urothelial cell lines. These data will guide the development and interpretation of future epidemiological studies investigating GST gene-environment interactions in canine TCC risk, and in developing chemopreventative strategies that might leverage this biotransformation pathway.

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Poster #51

EC-specific EphA4 ablation ameliorates BBB disruption, cortical damage and functional deficits following TBI

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Disruption of the blood brain barrier (BBB) is a major driver of secondary damage following traumatic brain injury (TBI) including edema, inflammation, and excitotoxicity. Although BBB dysfunction is a predictive marker for poorer outcomes and long-term disability, the cellular and molecular mechanism(s) regulating the BBB following TBI are not well understood. Recent evidence suggests Eph receptors, the largest family of receptor tyrosine kinases, contribute to various neurological insults. Using endothelial cell (EC)-specific EphA4 knockout (KO) mice, we find a significant decrease in BBB disruption compared to wild-type (WT) mice at 6 hours, 1 day, and 4 days post-TBI. Based on these findings, we *hypothesize* that EphA4 signaling on ECs negatively regulates BBB integrity and neuro-restoration following injury. To test our hypothesis we evaluated motor behavior at 3, 7 and 14 days post-TBI then euthanized the mice to analyze brain tissue sections using immunohistochemistry. Our findings indicate that EC-specific ablation of EphA4 significantly reduces lesion volume compared to WT at 1, 4, and 14 day post-CCI injury. This correlated with a significant increase in motor recovery, using Rotarod and beam walk assessments. Further studies will address the mechanistic role of EphA4 on BBB disruption, including the bi-directional communication between ECs and astrocytes in the neurovascular niche. This data provides evidence of a novel negative regulator of the EC response following TBI that may be exploited for therapeutic purposes.

Research Grant: This work was support by the NIH; NINDS R01NS096281 (MHT)
Student Support: Virginia-Maryland Regional College of Veterinary Medicine dual degree program

Poster #52

Neutrophil localization and interactions in the spleen of a mouse model of systemic lupus erythematosus

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Abstract: Systemic Lupus Erythematosus (SLE) is a complex autoimmune disorder that affects humans, canines, and rarely felines and equines. SLE manifests with a constellation of clinical signs and is driven by antigen-autoantibody complex deposition. It is generally thought of as an adaptive immune system disease, however recent work has elucidated dysregulation of the innate immune system as well. Neutrophils are the primary innate immune defenders against pathogens and are commonly short-lived and rapidly recruited to sites of inflammation. Recent studies have demonstrated significant plasticity in their behavior, particularly in SLE. Neutrophil numbers are increased in the spleen of SLE patients and SLE mouse models, and these neutrophils secrete cytokines such as BAFF and IL-17 that can regulate B and T cell function as well as act in an autocrine fashion. Neutrophils are found in both the marginal zone and T cell zone of the white pulp, with variable localization depending on the model and disease course. We are investigating potential alterations in the phenotype and function of splenic neutrophils in the MRL/MpJ-*Fas*^{lpr} SLE mouse model. We have found increased numbers of neutrophils that localize to the red pulp and T cell zone during active disease. Flow cytometric and image analysis of this neutrophil population shows increased IL-17 and IL-1β expression, MHC-II expression, and direct interaction with T cells, while other canonical neutrophil functions (ROS production, phagocytosis) are not altered. Assessment of neutrophil chemotaxis is ongoing. Further work is necessary to elucidate the role this unusual neutrophil population plays in the pathogenesis of SLE.

Research Grant: Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine

Student Support: The Stamps Foundation, Virginia-Maryland College of Veterinary Medicine

Poster #53

Johne's disease-investigation beyond the organism

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Abstract:
Johne's disease (JD), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a chronic inflammatory intestinal disease of wild and domestic ruminants worldwide. Infection results in severe weight loss, diarrhea, and decreased milk production. In the United States this disease has devastating economic impact, estimated at $200 million annually. Currently there are no vaccines, treatments, or control strategies capable of preventing disease, due in part, to a lack of understanding the mechanisms of protective immunity. Genome wide association studies (GWAS) have identified resistance associated polymorphisms in Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), Solute carrier family 11 member 1 (SLC11A1), and Vitamin D receptor (VDR) genes. The products of NOD2, SLC11A1, and VDR genes function together in the innate immune response to Mycobacteria, and thus polymorphisms in these genes can potentially influence the host response to infection. Our lab has identified a potentially resistant breed of sheep originating from Tamil Nadu, southern India. Although diagnostically positive for JD, upon necropsy these animals lacked both gross and histopathological characteristics of disease. We hypothesize that susceptible sheep will exhibit increased granulomas and pathological damage characteristic of JD compared to resistant sheep.

Research Grant: Virginia Maryland College of Veterinary Medicine
Student Support: DVM/PhD dual degree support

Poster #54

Rough *Brucella* expressing GnRH and FSH: A novel brucellosis immunocontraception vaccine

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While brucellosis has been eradicated from domestic livestock in the United States, the causative agent is still present in elk, bison, and feral swine. The interaction between infected wildlife, domestic livestock, and humans poses a great health risk. Of particular concern, feral swine populations have quadrupled in the past ten years and continue to expand nationwide. Feral swine are known carriers of brucellosis and other zoonotic diseases like leptospirosis, pseudorabies, *E. coli* O157:H7, and swine influenza. The current population control practices have neither minimized their spread nor the conservative $1.5 billion dollars of damage a year to agriculture they cause. There is a need to efficiently control the feral swine population and prevent the spread of zoonotic diseases, like brucellosis, to domestic food animals and ultimately the public. Two rough strains of *Brucella* (*B. abortus* RB51 and *B. neotomae*) expressing gonadotropin releasing hormone (GnRH) and/or follicle stimulating hormone (FSH) have the potential to be an effective immunocontraceptive for feral swine management, while reducing the spread of brucellosis. These strains could pave the way for novel effective immunocontraceptive tools to be used in wildlife and domestic animal health management.

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