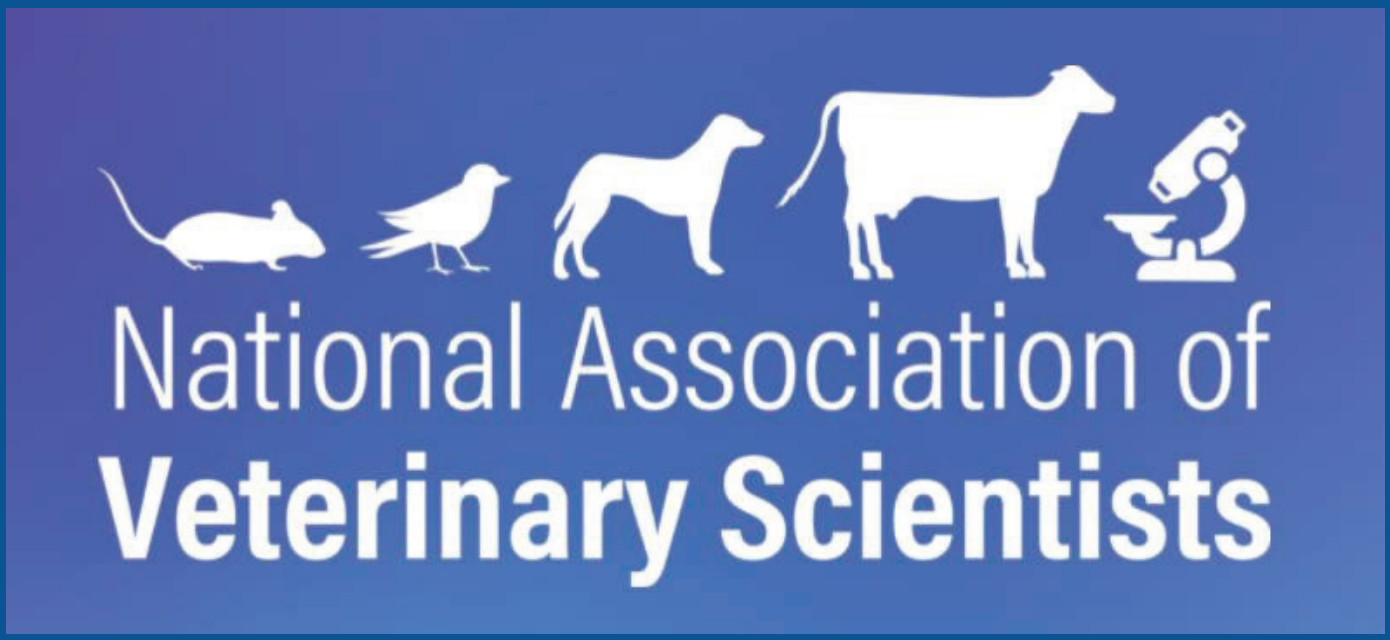

5th Annual Combined Degree Colloquium
August 3rd-4th 2021

Program Directory

5th Annual Combined Degree Colloquium

Presented by the National Association of Veterinary Scientists

Tuesday August 3, 2021

1:00 - 2:00 pm EDT
Keynote Address
K. Paige Carmichael
UGA

2:15 - 3:15 pm EDT
Oral Research Presentations Pt 1
Kelsey Murphy (VA-MD)
Amanda Loehr (Cornell)
Juselyn Tupik (VA-MD)

3:30 - 4:30 pm EDT
Current Student Panel *
Webinar + Breakout Rooms

Wednesday August 4, 2021

1:00 - 2:30 pm EDT
Alumni Panel *
Matt Kuhn (AAAS)
Robin Holland (USDA)
Sara Thomasy (UCD)
Frances Chen (Loyal)
Webinar + Breakout Rooms

3:00 - 4:00 pm EDT
Combined Degree Poster Session *
iPoster + Breakout Rooms

4:15 - 5:15 pm EDT
Oral Research Presentations Pt 2
Eileen Troconis (Cornell)
Ankita Gupta (NCSU)
Laurel Haines (CSU)

5:15 - 6:30 pm EDT
Happy Hour! *
Breakout Rooms Only

Please join us at the webinar:
https://ncsu.zoom.us/s/99099573977

All events will be hosted over Zoom Webinar, unless otherwise noted. Zoom links for breakout rooms will be shared on the day of.

Thanks to our sponsors for their continued support!

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AAVMC
American Association of Veterinary Medical Colleges

Keynote Biography

Dr. K. Paige Charmichael

Professor of Veterinary Pathology, University of Georgia College of Veterinary Medicine

Dr. K. Paige Carmichael is Professor of Veterinary Pathology in The University of Georgia College of Veterinary Medicine (UGA CVM). She served as the UGA CVM's Associate Dean for Academic Affairs from 2006-2015. Dr. Carmichael received her Doctor of Veterinary Medicine degree from Tuskegee University School of Veterinary Medicine (TUSVM) in 1987. She earned a combined pathology residency/Ph.D. at UGA in 1993, and became Board Certified in Veterinary Anatomic Pathology in 1995.

She has been awarded numerous teaching awards including the Lilly Teaching Fellowship, the Norden-Pfizer Teaching Award, and the Tyler Award for Teaching Innovation. She was inducted into the University's Teaching Academy in 2005, and in 2006, she became the first African American professor at UGA to receive the Josiah Meigs Distinguished Teaching Professorship. She is one of the founders and coordinators of the UGA Teaching Academy Fellows program.

Dr. Carmichael's area of research is animal models of human disease, and she has studied and published on many diseases common to people and animals. She is one of only a few veterinary ocular pathologists and runs the UGA Diagnostic Ocular Pathology Service. She is passionate about diversifying the veterinary profession and mentoring and "pathfinding" for students and early career faculty. She is the founder of the UGA CVM's VetCAMP and the Dog Doctors Outreach program. In 2013, she was honored with the TUSVM Distinguished Alumnus Award, and in 2014 she was awarded the LGVMA Outstanding Achievement Award and subsequently joined the Board of this organization. In 2015, she received the National Iverson Bell Award for her role in leadership in diversifying the veterinary profession in 2019, she was one of three people awarded the University of Georgia's President's Fulfilling the Dream Award for her diversification and mentoring efforts. She was also awarded the Lothar Tresp Outstanding Honors Professor Award, becoming the first professor from the College of Veterinary Medicine to be so honored. In 2019, she was the first African American to be honored as a Distinguished Member of the American College of Veterinary Pathologists (ACVP) for her contributions to diversity and her mentoring of veterinary students and pathology residents and most recently, in 2021, she was elected to the Board of Directors of the ACVP.

Alumni Panelist Biographies

Matt Kuhn, DVM, Ph.D.

AAAS Science and Technology Policy Fellow

After finishing his PhD in bovine immunology in the summer of 2020, Matt transitioned into a AAAS Science and Technology Policy Fellowship where he supports the Department of Defense. In this role, Matt acts as an editor and outreach coordinator for a peer-reviewed journal of controlled and classified federal research. In September, he will begin his second year in the program by moving to the Department of Homeland Security's Countering Weapons of Mass Destruction Office to work on a Bio-defense portfolio. Matt has had an interest in science policy since early in veterinary school and continues to seek ways to influence public policy for the betterment of animals and the veterinary industry. He publishes a quarterly blog that tracks and distills animal and public health legislation in Michigan, sits on the Michigan Veterinary Medical Association's Legislative Advisory Committee, and currently co-chairs the One Health Affinity Group for the AAAS STPF program.

Robin Holland, DVM, Ph.D.

Foreign Animal Disease Diagnostic Laboratory, Plum Island Animal Disease Center, USDA

Robin Holland is the Head of the Diagnostics Services Section at the Foreign Animal Disease Diagnostic Laboratory of the Plum Island Animal Disease Center. She received her Bachelor of Science in Agriculture from Murray State University in Murray, Kentucky, and a DVM and PhD in the Veterinary Medical Scholars Program at the University of Illinois at Urbana-Champaign, where she also set up a high-throughput COVID-19 diagnostic lab. Robin has experience in diagnostics and high biocontainment facilities, including the Friedrich Loeffler Institute in Greifswald, Germany, and the US Army Medical Research Institute of Infectious Diseases in Frederick, Maryland. Currently, Robin oversees all diagnostic testing of foreign animal disease investigations at FADDL, and she works closely with colleagues and partners nationally and internationally to increase preparedness and diagnostic response to major transboundary and emerging infectious disease incursions.

Sara Thomasy, DVM, Ph.D., DACVO

Department of Surgical and Radiological Sciences, School of Veterinary Medicine, U.C. Davis

Dr. Sara Thomasy is a Professor in the Department of Surgical and Radiological Sciences in the School of Veterinary Medicine at the University of California, Davis. She received her B.S. in Biology from The Ohio State University in 2000 and her DVM from UC Davis in 2005. She then completed a PhD in pharmacology and toxicology from UC Davis in 2006. Following a 1-year small animal rotating internship at North Carolina State University, she completed a comparative ophthalmology residency at UC Davis in 2010. Dr. Thomasy is a Diplomate of the American College of Veterinary Ophthalmology and serves as a reviewer for several journals including Investigative Ophthalmology and Vision Science, Acta Biomaterialia and Ophthalmology. She is a core scientist at the California National Primate Research Center and co-runs a large, interdisciplinary vision science laboratory with Drs. Christopher Murphy, Paul Russell and Brian Leonard. Her research interests include large animal models for anterior segment disease, corneal wound healing, glaucoma, ocular pharmacology, and antiviral therapy for the management of feline herpesvirus.

Frances Chen, DVM, Ph.D.

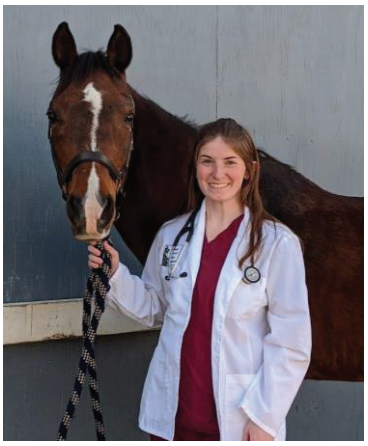
Loyal, Head of Veterinary Translational Medicine

Frances completed the Cornell DVM/PhD program in 2020 (PhD ’18, DVM ’20), where she utilized CRISPR/Cas9 genome editing in mice to investigate the function of noncoding regulatory elements in organ development and implicated a role for a novel long noncoding RNA in atrial arrhythmias. Upon return to the DVM curriculum, Frances performed internships with the Working Dog Project under Elinor Karlsson’s lab at the Broad Institute, Embark Veterinary, and Behavior Vets of NYC. After deliberating with trusted mentors and connecting with established alumni in Animal Health, Frances decided not to pursue an academic postdoc and accepted an offer to join the founding team at Cellular Longevity, Inc dba Loyal. As Head of Veterinary Translational Medicine, she oversees the design and execution of observational and interventional studies of aging and age associated disease in companion dogs. Frances works cross functionally across the R&D and Clinical Operations teams to identify, develop, and validate comparative aging biomarkers and clinical endpoints in proof of concept dog studies. One of her favorite parts of this role is aligning incentives to foster collaboration with veterinarian-scientists and clinicians across private veterinary clinics and academic institutions. Frances currently resides in the San Francisco Bay Area. When not company building, she spends most weekends exploring trails with her 10.5 year old released Guiding Eyes for the Blind dog Rooney, appreciating access to good food, connecting in person and remotely with other veterinarian scientists, and generally enjoying life outside of Ithaca.

Student Panelist Biographies

Ashley Putman

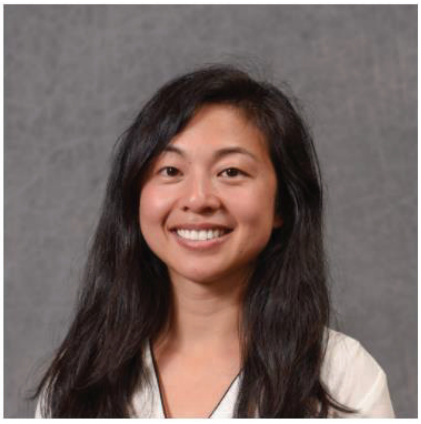
Michigan State University



Ashley is a sixth year DVM/PhD student at Michigan State University and the Director Contact of the NAVS colloquium planning team. Her dissertation research focuses on the physiological role of isoprostanes, a product formed during lipid membrane damage, in models of acute inflammation. Outside of lab, Ashley enjoys equestrian activities, exploring nature with her dog, and keeping up with the latest movies and TV shows.

Annie Wang

North Carolina State University



Annie is the NAVS Coordinator and is a colloquium planning team member. She studies the ecology, evolution, and epidemiology of antimicrobial resistance in enteric bacteria using nontraditional computational methods.

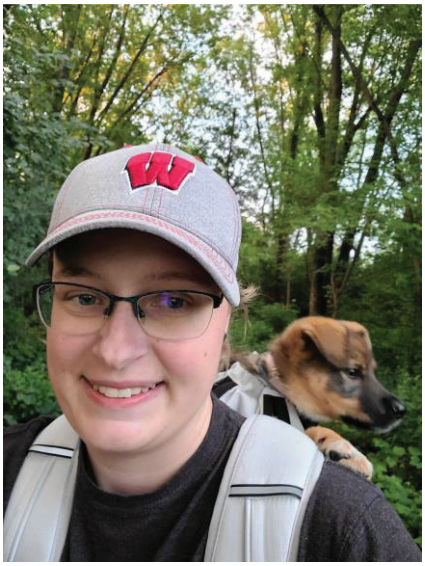
Katherine Griffin

University of California Davis

Katherine is the NAVS secretary and a colloquium planning team member. She studies the role of tissue resident macrophages and immunoengineering in various musculoskeletal diseases. Katherine is particularly interested in immunomodulation for improved regenerative medicine outcomes.

Hannah Ruetten

University of Wisconsin



Hannah Ruetten is a DVM/PhD candidate in Chad Vezina's Lab at University of Wisconsin- Madison. She completed her Bachelor's degree in Biology with Biomedical emphasis at University of Wisconsin- River Falls and started the dual DVM/PhD degree program at University of Wisconsin-Madison in 2014. Earlier in her PhD training she was a TL1 trainee and completed a certificate in Fundamental of Clinical Research through UW-Madison's Institute for Clinical and Translational Research.

She currently holds an F30 Fellowship position (awarded by the National Institute of Diabetes and Digestive and Kidney Diseases). Ms. Ruetten has published nine first-author manuscripts during her time as a PhD student. Her research interests include optimizing research models for lower urinary tract dysfunction, evaluating how key clinical factors such as inflammation timing and chronicity contribute to voiding dysfunction, and determining key pathways and cells involved in prostatitis and pathologic prostatic collagen accumulation.

She also enjoys teaching and mentoring and completed a DELTA Teaching Certificate which included an internship where she explored tactics to decrease cognitive overload within the veterinary curriculum. She has completed all of the requirements for her PhD, and, in Fall 2021, Ms. Ruetten will return to the DVM curriculum to complete the DVM portion of her combined degree. Her ultimate goal is to obtain a tenure track research position at a research institution.

Student Oral Presentations

Tuesday, August 3rd

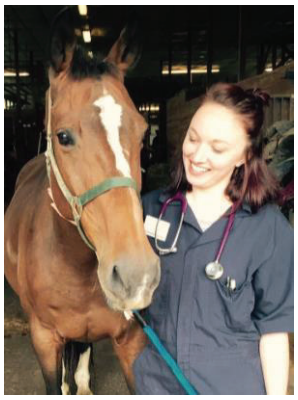
Kelsey Murphy

Virginia-Maryland College of Veterinary Medicine

Kelsey is originally from New Hampshire, and attended St. Lawrence University where she earned her Bachelor's degree in Chemistry. She now attends Virginia-Maryland College of Veterinary Medicine as a dual DVM/Ph.D. student. Within the Biomedical and Veterinary Sciences Department of the vet school, Kelsey works with Dr. Nikolaos Dervisis at the Animal Cancer Care and Research Center studying tumor ablation therapies and their application for canine and human brain tumor ablation.

Amanda Loehr

Cornell University



Amanda is a fifth year DVM/PhD Combined Degree student at Cornell University in the lab of Dr. Robert Weiss. She studies the developmental origins and unique chemosensitivity of testicular germ cell tumors. In her free time, Amanda loves to cook, read a good book, and explore the outdoors with her three-legged dog, Bean.

Juselyn Tupik

Virginia-Maryland College of Veterinary Medicine

Juselyn is an upcoming 3rd year DVM/Ph.D. student at the Virginia-Maryland College of Veterinary Medicine. Her dissertation project in Dr. Coy Allen's lab focuses on understanding the NOD-Like Receptor (NLR) innate immune response to the bacterial diseases Brucellosis and Lyme Disease. She hopes to utilize her combined degree to pursue research and laboratory animal medicine.

Wednesday, August 4th

Eileen Troconis, M.S.

Cornell University

Eileen was born in Merida, Venezuela. She received her bachelor's degree in Biology from Amherst College, and then completed a master's degree in Veterinary Science at the University of Cambridge. Eileen is currently a DVM-PhD student in the Warden lab at Cornell University. Her thesis work looks at the activity of the female brain serotonin system during mating and other behaviors.

Ankita Gupta

North Carolina State University

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

Laurel Haines

Colorado State University

Laurel Haines grew up in Ashby, Massachusetts. She pursued an undergraduate degree at the University of Vermont where she studied how traumatic brain injury can damage the systemic vasculature. Before joining the DVM/PhD program at Colorado State University, Laurel spent several years helping to develop vaccines for *C. difficil*e and *S. pneumoniae* at Matrivax R & D, a vaccine development company in Boston, MA. Currently, Laurel is entering the third year of the DVM/PhD program at CSU where she researches cancer metastasis in Dr. Daniel Regan's laboratory. Her Ph.D. project is focused on how a primary tumor can prime a distant organ to allow the seeding and outgrowth of circulating tumor cells resulting in metastasis. In her free time, Laurel enjoys making pottery and spending time in the Rocky Mountains skiing, rock climbing, and hiking with her dog Elisa.

Investigation of blood-brain barrier disruption induced by high-frequency irreversible electroporation brain tumor ablation

Murphy, Kelsey R.[1,2]; Aycock, Kenneth N.[3]; Hay, Alayna N.[1]; Davalos, Rafael V.[3]; Rossmeisl, John H.[1,2]; Dervisis, Nikolaos G.[1,2]

[1] Animal Cancer Care and Research Center, Virginia-Maryland College of Veterinary Medicine, Roanoke, Virginia, USA.
[2] Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, Virginia, USA.
[3] Department of Biomedical Engineering and Mechanics, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Malignant brain tumor treatments are hindered by the blood-brain barrier (BBB), which shelters neoplastic cells. High-frequency irreversible electroporation (H-FIRE) is a minimally-invasive, nonthermal ablative therapeutic in clinical trials for canine primary brain tumors. H-FIRE precisely ablates brain tumors while transiently disrupting peritumoral BBB, enhancing therapeutic delivery to invasive tumor margins. The mechanisms of H-FIRE-induced cell death and BBB disruption remain incompletely characterized. We hypothesize that H-FIRE-induced brain tumor cell death indirectly induces BBB disruption.

F98 glioma, LL/2 Lewis lung carcinoma, and bEnd.3 cerebral endothelial cell lines modelled primary and metastatic brain cancer and BBB endothelium, respectively. Cell suspensions were treated with 200 bursts of 2-5-2 μs bipolar pulses, with electric fields escalating to 3,000 V/cm. Cell membrane permeability and chromatin condensation of cancer cells were temporally measured via flow cytometry. Endothelial cells were exposed to dose-escalating H-FIRE-treated cancer cell supernatants. Effects of endothelial cell exposure to H-FIRE-treated cancer cell supernatant were assessed via quantitative RT-PCR for tight junction-associated gene expression and analysis of Annexin V/PI via flow cytometry to quantify apoptosis.

Both cancer cell lines exhibited voltage-dependent permeabilization and chromatin condensation, with recovery of membrane integrity and chromatin organization occurring at lower voltages. Chromatin condensation post-treatment suggests a role of apoptosis in H-FIRE-induced cell death. Exposure of cerebral endothelial cells to H-FIRE-ablated cancer cell-conditioned supernatant resulted in rapid detachment and disruption of normal endothelial morphology that was not induced by apoptosis, suggesting an indirect mechanism of BBB disruption following brain tumor treatment with H-FIRE.

Targeting cancer stem cells with differentiation agents for the treatment of malignant testicular germ cell tumors

Loehr, Amanda R.; Pierpont, Timothy M.; Gelsleichter, Eric; Galang, Anabella Maria D., Fernandez, Irma R.; Moore, Elizabeth S.; Guo, Matthew Z.; Miller, Andrew D.; and Weiss, Robert S.

Department of Biomedical Sciences, Cornell University, Ithaca, NY, USA

Testicular germ cell tumors (TGCTs) are exceptionally sensitive to conventional genotoxic chemotherapy, resulting in a high cure rate for the young men presenting with these malignancies. However, this treatment is associated with significant toxicity, and a subset of malignant TGCTs demonstrate chemoresistance. Mixed non-seminomas, a subtype of TGCTs, often contain pluripotent embryonal carcinoma (EC) cells, the cancer stem cells (CSCs) of these tumors. We hypothesized that differentiation therapy, a treatment strategy which aims to induce differentiation of tumor-propagating CSCs to slow tumor growth, could effectively treat mixed non-seminomas without significant toxicity. The FDA-approved anti-psychotic thioridazine and the agricultural antibiotic salinomycin are two drugs previously found to selectively target CSCs in other cancers, and here we show that these agents differentiate EC cells in vitro and greatly reduce their tumorigenic potential in vivo. Using a novel transformed induced pluripotent stem cell allograft model, a human embryonal carcinoma xenograft model, and a genetically engineered mouse model featuring metastatic TGCTs, we show that thioridazine extends the survival of tumor-bearing mice and can reduce the number of pluripotent EC cells within tumors. These results suggest that thioridazine or related compounds could be utilized as alternative TGCT treatments that avoid the toxicity of conventional chemotherapeutics, by targeting only the tumorigenic EC cells within these tumors.

A “sub-Lyme” system: Uncovering the protective role of anti-inflammatory NLRX1 against Lyme disease

Tupik, Juselyn D.[1]; McClune, Mecaila E.[2]; Dressler, Julianne M.[2]; Jutras, Brandon L.[2]; Allen, Irving C.[1,3]

[1] Department of Biomedical & Veterinary Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061
[2] Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061
[3] Department of Basic Science Education, Virginia Tech Carilion School of Medicine, Roanoke, VA, 24016

Lyme disease, caused by the bacterium *Borrelia burgdorferi*, is an enigmatic disease of pressing concern in the field of immunology. Upon transmission, the bacterium promotes chronic inflammation of the host’s joints inducing Lyme arthritis, which affects roughly 475,000 individuals in the United States per year. Immune processes underlying the initiation and mitigation of this inflammation have not been fully elucidated, making it important to define these mechanisms to develop therapies for Lyme disease. Pattern Recognition Receptors (PRRs) serve as the first line of host innate immune defense against pathogens and operate by sensing conserved genetic patterns known as Pathogen-Associated Molecular Patterns (PAMPs). Intracellular PRRs known as the NOD-Like Receptors (NLRs) have been implicated in the recognition of *Borrelia,* with pro-inflammatory NLRs instigating inflammation-promoting Lyme arthritis. However, the role that anti-inflammatory NLRs play in mitigating this inflammation has not been elucidated.

Here, we studied the role of anti-inflammatory NLRX1 during Lyme disease by using novel knockout mouse models. We infected wildtype and *Nlrx1*$^{-/-}$ mice with a moderate dose of 10^5 *Borrelia burgdorferi* spirochetes for 30 days. We found that *Nlrx1*$^{-/-}$ mice exhibited significantly earlier, more severe arthritis than wildtype mice, which suggests that NLRX1 serves to attenuate inflammation during Lyme arthritis **(Figure 1)**. We further investigated potential *Borrelia* PAMPs, including the outer biopolymer layer of the bacterium called peptidoglycan (PG) and genomic (g)DNA. We introduced *Borrelia* PG to these mouse models and found that *Nlrx1*$^{-/-}$ mice exhibited elevated arthritis compared to wildtype. Additionally, we found that *Nlrx1*$^{-/-}$ macrophages showed increased inflammatory cytokines in response to *Borrelia* gDNA. These results indicate that *Borrelia* PG and gDNA could serve as effective *Borrelia* PAMPs for NLRX1. Ultimately, these results indicate that NLRX1 plays a protective role in mitigating Lyme arthritis, warranting a further need to define NLRX1’s mechanism of activation during Lyme disease.

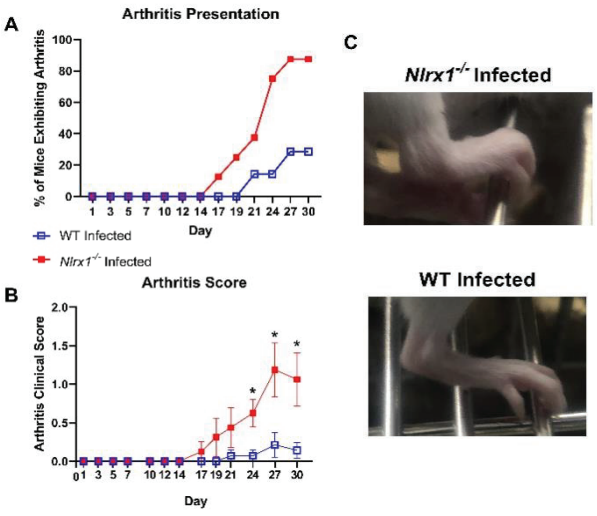


Figure 1: NLRX1 inhibits *Borrelia burgdorferi* arthritis presentation
12-week old Balb/c WT (n=7) and *Nlrx1*$^{-/-}$ (n=8) mice were infected IP with 10^5 cells of *Borrelia burgdorferi* strain B31. *Nlrx1*$^{-/-}$ mice exhibited significantly elevated **A)** % Arthritis presentation [87.5% to 28.5% at 30 d.p.i] and **B)** Arthritis clinical score [1.19 to 0.21 at 30 d.p.i] over wildtype mice. **C)** Representative photos 29 d.p.i. of arthritis of the tarsal joint of infected *Nlrx1*$^{-/-}$ and WT mice.

Female dorsal raphe serotonin responses to sexual stimuli

Troconis, Eileen L.[1]; Seo, Changwoo[1]; Guru, Akash[1]; Miller, Caitlin H.[1]; Vogt, Caleb C.[1]; Warden, Melissa R.[1]

[1]Department of Neurobiology and Behavior, Cornell University, Ithaca, NY, U.S.A.

Brain serotonin—a neuromodulatory system with widely-distributed projections to the forebrain—has been implicated in a variety of emotional brain states and behaviors, including mating. Previous work in both humans and animal models has shown that pharmacological compounds that interact with the serotonin system can modulate female sexual behavior. However, whether female serotonin neurons normally respond to sexual stimuli during mating is unknown. Here, we used fiber photometry to record population calcium activity of dorsal raphe serotonin neurons in the female mouse brain during mating. We found that this neuronal population becomes active in the female at the time of ejaculation. To understand which stimuli during ejaculation trigger this neuronal activity in the female, we developed surgical techniques that perturb various aspects of ejaculatory physiology in males with intact mating behavior. First, we tested the role of ejaculatory fluid release into the vaginal canal by redirecting the male urethra to a stoma in the abdominal wall. Then, we tested the role of penis erectile function by partially transecting a pair of muscles that control penis behavior. We found that the female elevated serotonin neuronal activity at ejaculation disappeared only when her mating partner had undergone both modifications in combination. Moreover, artificial mechanical vaginal stimulation was sufficient to trigger serotonin neuronal activity in the female. Together, these results suggest that both penis behavior and release of ejaculatory fluid inside the vaginal canal are important mechanical stimuli to which female dorsal raphe serotonin neurons respond during ejaculation. Detecting ejaculation may be crucial for regulating various aspects of female sexual behavior and physiology, such as receptivity in subsequent mating encounters and fertility. Overall, our work sheds light on a novel vagina-brain axis that likely plays a critical role in female reproduction.

Artemin/GFRα3 signaling axis is involved in the functional plasticity of sensory neurons in OA-pain

Gupta, Ankita[1,2]; Nair, Uma[1]; Mishra, Santosh K.[3,4]; Lascelles, B. Duncan X.[1,2,4,5,6]

[1]Translational Research in Pain Program, North Carolina State University, Raleigh, NC, U.S.A.
[2]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, U.S.A.
[3]Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, U.S.A.
[4]Comparative Pain Research and Education Centre, North Carolina State University, Raleigh, NC U.S.A.
[5]Thurston Arthritis Center, University of North Carolina, Chapel Hill, NC U.S.A.
[6]Center for Translational Pain Medicine, Duke University, Durham, NC, U.S.A.

Osteoarthritis (OA) is a leading cause of disability, with ~100 million people in the US suffering from chronic joint pain and resulting widespread sensitization and decreased mobility. Clinically efficacious and safe therapeutic options for OA-pain management are limited. *Thus, there is an urgent need to develop novel, clinically relevant analgesics for OA-pain.* Recently, we found increased concentrations of a neurotrophic factor, artemin, in the serum of dogs, cats, and humans with naturally occurring OA-pain. Further, GFRα3 expression (artemin's receptor) was increased in canine OA sensory neurons compared to controls. *Despite our compelling data, no studies have elucidated the role of artemin/GFRα3 signaling in the development and maintenance of OA-pain.* Our current work employs cellular and molecular approaches to identify (i) the source of artemin's release in the periphery, (ii) changes in sensory neuron colocalization of GFRα3 and downstream "pain channels", such as transient receptor potential (TRP) ion channels, and (iii) the functional role of ARTN/GFRα3 signaling in chronic OA-pain. We hypothesize that ARTN released from damaged OA joint tissues results in both functional upregulation and *de novo* expression of GFRα3 in sensory nerves, producing pain via TRP channel upregulation. Here, we utilized the monoiodoacetate mouse model to show robust reversal of lameness after systemic injection of anti-artemin monoclonal antibody. This is the first evidence investigating the functional role of artemin/GFRα3 signaling in chronic OA-pain and limb use. Our ongoing work explores which peripheral joint tissues release artemin and changes in GFRα3 and TRP channel expression in sensory neurons serving OA joints (**Figure**). Overall, our work will elucidate the role of artemin/GFRα3/TRP signaling in OA-pain and define putative targets for developing safe and effective treatments.

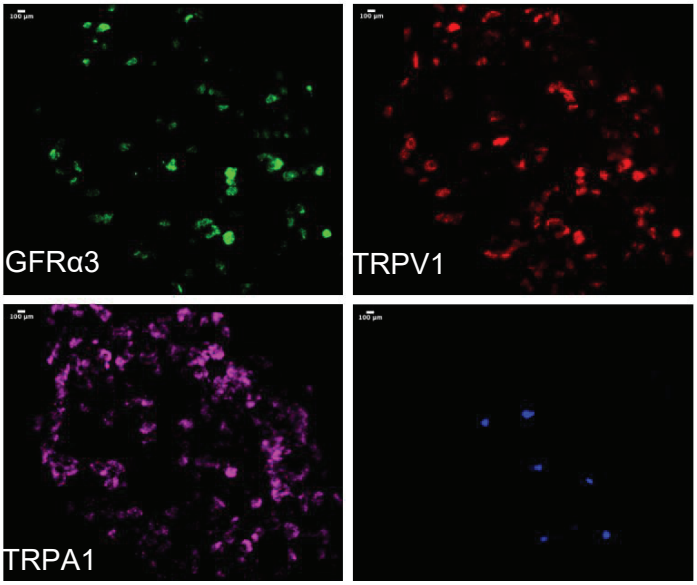


Figure: Distribution of GFRα3, TRPV1 (hot), TRPA1 (noxious cold), and TRPM8 (innocuous cold) in murine OA sensory neurons. 20x magnification. Labelled with RNAscope in-situ hybridization.

Osteosarcoma exosomes selectively home to the lung and elicit pro-tumorigenic changes in resident lung cell populations

Haines, Laurel A.[1]; Schofield, Sophi J.[1]; Palmer, Eric P.[1]; Cronise, Kathryn E.[1]; Andretsos, Chris G.[1]; Regan, Daniel P.[1]

[1]Department of Microbiology, Immunology, and Pathology, Colorado State University College of Veterinary Medicine, Fort Collins, CO, U.S.A.

Osteosarcoma (OS), the most common primary malignant tumor of bone, often progresses to a highly fatal metastatic disease with limited treatment options. Following resection of the primary tumor, one-third of OS patients relapse with metastases, almost exclusively in the lung. Metastasis is preceded by the formation of a pre-metastatic niche, a process by which distant sites in the body are “primed” for tumor cell seeding by factors secreted by the primary tumor. Of these secreted factors, nano-sized extracellular vesicles, also known as exosomes, have been shown to be crucial mediators of pre-metastatic changes in resident host cells, and display highly specific organotropism in certain metastatic cancers. Little is known about the role of OS exosomes in modulating the pulmonary microenvironment during OS metastasis. We hypothesize that OS exosomes selectively home to the lung and instruct resident lung cells to create a pro-metastatic microenvironment characterized by an anti-inflammatory immune profile and dysregulated extracellular matrix composition. To investigate this, we evaluated human OS exosome biodistribution and cellular uptake in mice using intravital imaging, flow cytometry, and immunofluorescence. We also investigated the immunological effects of OS exosomes *in vivo* in mice and in primary human donor-derived lung fibroblasts and alveolar macrophages. We show that OS exosomes selectively track to the lung in mice and elicit distinct changes in known tumor-promoting cytokines both *in vivo* and *in vitro*. Our findings demonstrate that OS exosomes can alter the lung microenvironment prior to circulating tumor cell arrival. These pro-tumorigenic changes may promote metastasis during OS and could serve as early indicators and potential therapeutic targets for patients with metastatic disease.

Student Poster Presentations
August 4th

2021 Combined Degree Colloquium Poster Session Groups

3:00-4:00 pm EDT on Wednesday August 4, 2021

The Combined Degree poster session will take place on Wednesday August 4, 2021 from 3:00-4:00 pm EDT. During our poster session, attendees will have the opportunity to interact with student poster presenters in breakout rooms. From 3:00-3:30 pm EDT, roughly half of the student poster presenters will be available in their assigned breakout rooms and the other half will be available from 3:30-4:00 pm EDT. Breakout rooms are organized by research topic area and described below. Prior to the poster session, we highly encourage attendees to view the posters in iPoster and come prepared with questions. Please note, iPoster access requires prior registration to NVSS in addition to the Combined Degree Colloquium.

	1st Half *3:00-3:30 pm EDT*	**2nd Half** *3:30-4:00 pm EDT*
Breakout Room 1	***Epidemiology 1*** ● Lilli Heinen ● Ashlan Jolley ● Elizabeth Ashley ● Makda Asrat	***Epidemiology 2*** ● Olivia Cords ● Annie Wang ● Cristina Blanco
Breakout Room 2	***Immunology 1*** ● Juselyn Tupik ● Arpita Nayak ● Carol Baker	***Immunology 2*** ● Daniela Jimenez ● Josetta Adams ● Madison Myers
Breakout Room 3	***Oncology*** ● Jenna Cao ● Laurel Haines ● Aryana Razmara	***Immunology 3*** ● Elise Peauroi ● Hannah Martin ● Alexandra Kaloss
Breakout Room 4	***Genetics/Omics 1*** ● Lily Kim ● Sydney Womack ● Jayden McCall	***Genetics/Omics 2*** ● Emily Winn ● Victoria Tobin ● Ashley Rasys ● Julia Baker
Breakout Room 5	***Surgery/Wounds*** ● Shanice Harris ● Jaclyn Carlson ● Ariela Burk	***Neurology*** ● Seth Lieberman ● Eileen Troconis
Breakout Room 6	***Other*** ● Alexandra Hommer ● Erin Hisey ● Ariel Shepley-McTaggert ● Eleanor Pressman	***Pharmacology/Therapeutics*** ● Alexandra Chiusano ● Madison Caldwell ● Rachel Gagliardi ● Cassandra Barber

Development of highly specific chicken IgY- based immunoassays for the detection of Staphylococcal leukotoxins

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Staphylococcus aureus (*S. aureus*) causes severe infections such as necrotizing pneumonia, diabetic foot ulcer, and infective endocarditis by producing several redundant virulence factors such as cytotoxins and superantigens. Identification of specific virulence vectors is the key to develop effective therapeutics. Traditionally, mouse or rabbit IgG-based immunoassays have been used, however, non-specific binding of mouse or rabbit IgG to staphylococcal protein A (SpA) hindered accurate identification and quantification of virulence factors. Chicken Immunoglobulin Y (IgY) has unique fragment crystallizable (Fc) sequences that do not have an affinity to SpA and other mammalian Fc receptors ideal to be used for immunoassays. In this study, chicken IgY specific to staphylococcal leukotoxins were generated and applied for western blot and enzyme-linked immunosorbent assay (ELISA). We demonstrate that chicken IgY successfully detected staphylococcal leukotoxins from in vitro culture supernatants and mouse tissue homogenates prepared from subcutaneous *S. aureus* infection. ELISA showed high specificity and sensitivity of detecting less than 10 ng of staphylococcal leukotoxins. Chicken IgY-based immunoassays established in this study could be highly reliable, effective, and applicable detection tools to analyze specific virulence factors produced by *S. aureus* i*n vitro* and *in vivo*.

Molecular identification of arthropod vectors and pathogens using mobile, third-generation sequencing and real-time PCR technologies

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Changing weather patterns are influencing ecological landscapes globally, leading to changes in the abundance and geographic distribution of insect vectors of veterinary importance. As vector ranges expand, they are typically accompanied by pathogens that can negatively impact naive host populations, thus contributing to disease emergence. To keep pace with changing landscapes, biosurveillance methods must be nimble; providing meaningful data in a short period of time with limited *a priori* information. Third-generation nanopore sequencing platforms and other mobile, genomic technologies are perfectly poised to allow for real-time, field-based surveillance, information that can be leveraged to detect and confront emerging vector-borne disease outbreaks. Here, we used portable nanopore sequencing and RT-PCR methods to detect molecular evidence of arthropod-borne pathogens of veterinary importance from native genomic samples; specifically focusing on the surveillance of biting midges (genus *Culicoides*), to monitor for epizootic hemorrhagic disease virus (EHDV), an emerging pathogen of particular concern in Minnesota. CDC light traps were deployed in areas adjacent to ungulate housing and waste-collection zones where *Culicoides spp.* are most abundant. Insect trapping, sample preparation and data analysis is ongoing. We anticipate our results will inform vector-borne disease surveillance initiatives throughout the state and upper Midwest. Our preliminary nanopore sequencing results for mosquito surveillance have already identified several known mosquito vector species and *Dirofilaria immitis*, the causative agent of canine heartworm.

Messenger RNA treatment to induce expression of bovine cathelicidins for antimicrobial effect against respiratory disease pathogens

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Respiratory disease resulting from infection by viruses, bacteria, or a combination of both frequently causes sickness and death in animals and humans. In cattle, coronavirus and *Pasteurella multocida* are common contributors; related pathogens lead to respiratory disease in humans and other species. Various methods to prevent bovine respiratory disease have either been inadequately effective or have led to antimicrobial resistance. Better methods of prevention could improve animal and human health. A novel preventive approach is to activate production of innate antimicrobial molecules by messenger RNA (mRNA) transfection of the respiratory epithelium, to induce rapid protection against respiratory disease. Cathelicidins are a group of innate immune mediators produced by white blood cells and epithelial cells with direct antimicrobial, inflammatory, and chemotactic effects. Our overall objective is to transfect bovine epithelial cells with synthetic mRNA encoding bovine cathelicidin 2 and modified bovine cathelicidin 5 (Syn 1 of Sahoo et al.), and to measure the antibacterial and antiviral effects of the expressed cathelicidins against *P. multocida* and bovine coronavirus (BCoV). Transfection of bovine cells with cathelicidin mRNA resulted in peak expression at 24 hours as indicated by the linked reporter molecule NanoLuciferase. Ongoing work will define the conditions for optimal antimicrobial effect. This research will form the basis for future research to confirm efficacy in *in vivo* trials.

Nanopore-based adaptive sequencing for mosquito-borne disease surveillance

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Single-molecule nanopore sequencing is an emerging powerful tool for real time sequencing based biosurveillance and pathogen discovery. Recent software advancements have made it possible to selectively enrich for viral and bacterial communities without the need for host depletion protocols in an approach known as adaptive sequencing. This project utilized nanopore-based adaptive sequencing for surveillance of mosquito-borne pathogens in the Minneapolis and St. Paul areas of Minnesota. Mosquitos were collected with CDC light and gravid traps, from urban and forested ecosystems and identified using morphological characteristics and adaptive sequencing for molecular identification. DNA and RNA were sequenced for pathogen and strain identification.

An initial nanopore sequencing run was conducted using genomic DNA from 8 barcoded mosquitoes and generated over 3 billion base pairs in approximately 48 hours. Preliminary results indicate potential matches to mosquito species including *Culex pipiens*, *Aedes aegypti*, and *Culiseta alaskaensis*. *Dirofilaria immitis* (heartworm) was also potentially detected in the samples, and additional phylogenetic analyses will be performed to confirm these results. Future steps include additional mosquito collection and RNA analysis for pathogen discovery.

Canine osteosarcoma, a spontaneous large animal model for solid tumor CAR T cell therapy

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Chimeric Antigen Receptor (CAR) T cell therapy allows for the targeting of a specific surface antigens to generate an adaptive immune response. Use of CAR T cells targeting CD19 and CD20 have shown miraculous clinical success in treating advanced and relapse B cell malignancies. Application of CAR T cell therapy to solid tumors such as sarcomas and carcinomas have not shown the same clinical success, despite the identification of promising tumor antigens and successful preclinical data. The standard preclinical animal model utilizes the NOD SCID-gamma (NSG) mouse strain that is severely immune deficient which allows for human tumor xenografts and infusion of human CAR T cells. However, lack of functional myeloid lineage cells does not factor in the interaction of the tumor microenvironment (TME) of solid tumors on cellular antitumoral immunity, which is proposed as a major factor in the gap in translation from benchtop to clinical success. Tumor associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs) that reside within the TME exert immune suppressive pressure through cell signaling and secreted cytokines that dampen antitumoral cellular response. Osteosarcoma (OS) is an aggressive solid tumor that occurs in high prevalence in both adolescent children and large breed dogs. The checkpoint molecule B7-H3 has been identified in human (OS) as a marker correlated with poor prognosis, increased metastasis and decreased tumor infiltrating lymphocytes. **This study aims to develop canine osteosarcoma as a solid tumor model for pediatric osteosarcoma to evaluate the role of TAMs and MDSCs on CAR T cell efficacy targeting B7-H3 positive osteosarcoma.** We found that B7-H3 was over expressed in primary canine OS while normal liver and spleen had low to no expression of B7-H3. Level of B7-H3 correlated to canine B7-H3 CAR T cells activation as measured by secreted interferon gamma.

Collagen V haploinsufficiency results in delayed healing and altered wound matrix post-injury in murine tendons

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Patients with Classic Ehlers-Danlos syndrome (cEDS), a disorder characterized most commonly by *COL5A1* haploinsufficiency, suffer from tissue hyperelasticity, tendon/ligament fragility and abnormal wound healing. Collagen V (ColV) haploinsufficiency leads to abnormal tissue development and altered collagen assembly, and mechanical loading of the mouse patellar tendon shows a delay in healing and alterations in stiffness and dynamic modulus post-injury (PI). Therefore, the objective of this study was to determine the effect of ColV deficiency in female mice on wound matrix formation and resultant structure-function relationships when mechanical load is applied post-injury. We hypothesized that ColV deficiency will have effects post-injury, resulting in increased fibril diameter and cellularity, decreased mechanical properties and leading to a delayed healing response when compared to wild-type tendons. *Col5a1* expression was significantly increased in WT tendons at 1- and 3w PI compared to uninjured controls, with no significant changes in *Col5a1* expression seen PI in ***Col5a1***$^{+/-}$ tendons (Fig.1). TEM analysis showed ***Col5a1***$^{+/-}$ fibrils PI were larger and more broadly distributed than WT fibrils (Fig.2). Further, mechanical testing showed that WT tendons realigned through 5% strain, with ***Col5a1***$^{+/-}$ tendons continuing to realign through 6% strain (Fig.3A). Lastly, ***Col5a1***$^{+/-}$ tendons had a significant increase in cellularity persisting to 6w PI when compared to uninjured tendons, that was not seen in WT tendons (Fig.3B). Without the initial increase in ColV following injury, fibrillogenesis is less regulated, resulting in an altered fibril diameter distribution of ***Col5a1***$^{+/-}$ tendons PI. Additionally, increased cellularity in ***Col5a1***$^{+/-}$ tendons would alter the matrix alignment and architecture, weakening the tissue and affecting mechanical properties. This study indicates that the lack of an early increase in *Col5a1* expression PI in ***Col5a1***$^{+/-}$ tendons influences matrix architecture, alignment, and cellularity throughout tendon healing, demonstrating altered and delayed healing compared to WT tendons. *This study was supported by NIH/NIAMS AR065995 and the Penn Center for Musculoskeletal Disorders (AR069619).*

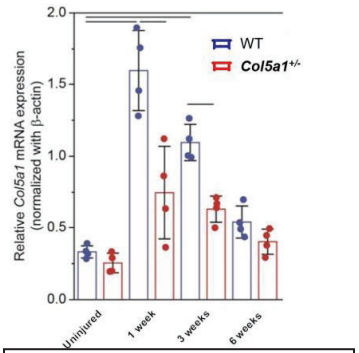

Figure 1: *Col5a1* expression increases 1w and 3w PI in WT but not in ***Col5a1***$^{+/-}$ tendons. Solid lines denote significance.

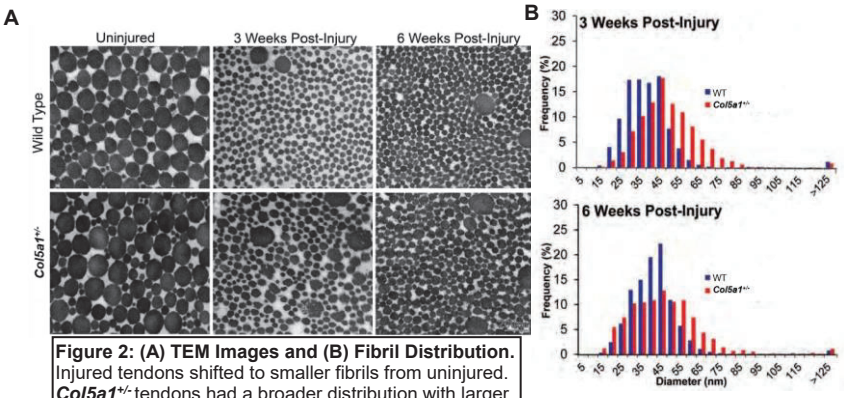

Figure 2: (A) TEM Images and (B) Fibril Distribution. Injured tendons shifted to smaller fibrils from uninjured. ***Col5a1***$^{+/-}$ tendons had a broader distribution with larger fibrils than WT tendons PI.

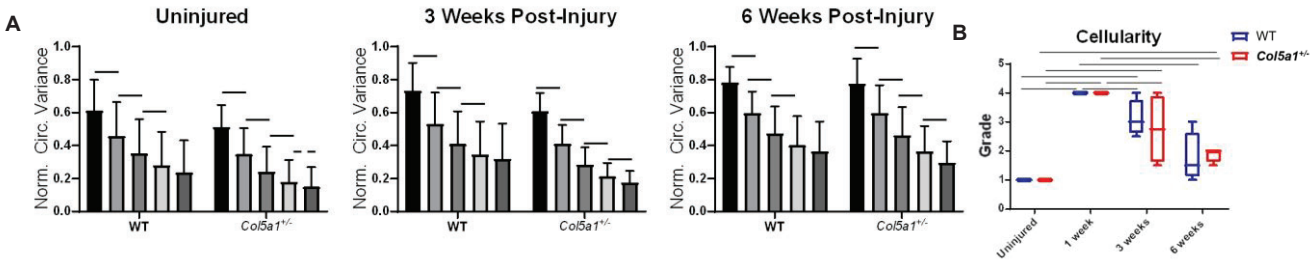

Figure 3: (**A) Midsubstance Fiber Realignment.** ***Col5a1***$^{+/-}$ uninjured and injured tendons realign through 6% strain, while WT realign through 5% strain. **(B) Cellularity.** Tendons of both genotypes increased significantly in cellularity 1 and 3w PI when compared to uninjured, this increase persisted to 6w PI in ***Col5a1***$^{+/-}$ tendons. Differences in cellularity were also seen between 1w and both 3 and 6w PI in both genotypes. Solid lines denote significance.

Establishing the role of the lateral habenula in central itch processing

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Itch is a complex sensory experience that also encompasses affective and behavioral components, as evidenced by the common presentation of comorbid depression, anxiety, and other psychological disorders in chronic itch patients. However, the cellular and circuit mechanisms linking pathological itch to affective disorders are poorly understood. Here, we identified a novel role for the lateral habenula (LHb), a predominantly glutamatergic brain region important for negative valence, aversive and avoidance behaviors, and depression, in mediating acute itch behavior. Using c-Fos immunohistochemistry in mice, we found that the LHb is highly activated by both itch and pain stimuli. To genetically access itch-activated LHb neurons, we used the *TRAP2* driver line (*Fos*^{iCreERT2}), which allows permanent expression of a Cre-dependent reporter allele in neurons activated by itch stimulation during a restricted time window. Anterograde tracing of itch-activated LHb neurons revealed projections to both the rostromedial tegmental nucleus and the dorsal raphe nuclei. Using optogenetic and chemogenetic approaches to reactivate itch-activated LHb neurons, we found that this population suppresses active behavioral responses to itch stimulation, such as scratching, and promotes passive immobility. Moreover, activation of these neurons or global chemogenetic inhibition of LHb neurons (using the *Vglut2*^{Cre} driver line) did not affect general locomotion, suggesting that the itch-evoked immobility behavioral response is context-specific. Activation also did not affect pain-related behaviors, raising the possibility that discrete LHb ensembles process different aversive sensory modalities. Preliminary evidence using chemogenetic inhibition of Vglut2^{+} LHb neurons suggests that this structure is required for the promotion of itch-evoked passive immobility, but does not appear to function as a brake on itch-evoked active scratching. Collectively, these results establish the LHb's role in promoting a passive behavioral state during acute itch challenge, and support a model in which the LHb mediates the development of depressive symptoms in association with chronic itch disorders.

The effect of BIO-PLY, a novel concentrated, fractionated, and pooled platelet-rich plasma lysate, on synoviocyte/chondrocyte co-culture supernatant viscosity

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Osteoarthritis is a debilitating disease that affects both humans and animals. It is a progressive disease that often results in significant pain, limited mobility, and reduces overall quality of life. Currently, there are no therapeutics in human or veterinary medicine that markedly alter the progression of OA and none that are able to reverse the negative effects of this disease. The purpose of this study was to investigate the effect of BIO-PLY, a novel concentrated, fractionated, pooled platelet-rich plasma lysate, on synoviocyte/chondrocyte co-culture supernatant viscosity. Previously, BIO-PLY has been shown to have significant anti-inflammatory properties and increase synoviocyte hyaluronic acid (HA) production. We hypothesize that supernatant from synoviocyte/chondrocyte co-cultures treated with BIO-PLY will have a greater viscosity than those untreated or those treated with conventional treatments (exogenous HA or steroid). Further, we hypothesize that the supernatant viscosity will increase over time (across our 24h, 48h, and 72h timepoints). In order to test these hypotheses, a co-culture system of donor matched equine synoviocytes and chondrocytes was utilized. Cells were stimulated with LPS to induce an OA-like phenotype. Treatments were implemented 24h post-LPS stimulation: no treatment control; exogenous HA (Hyvisc®); steroid (Kenalog®); and BIO-PLY. Samples were collected at 24h, 48h, and 72h. Supernatants were analyzed on a viscometer-rheometer on a chip (m-VROC, RheoSense) at flow rates in the range of 125µL/min – 200µL/min corresponding to their estimated viscosity. Apparent viscosity measurements were averaged to give an estimated apparent viscosity of each sample. Preliminary results demonstrate that BIO-PLY may increase the supernatant viscosity compared to traditional steroid and exogenous HA treatments as well as untreated controls. Further investigation is underway.

Characterization of antimicrobial peptides expressed by the equine ocular surface and amniotic membrane

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The ocular surface is an important interface with the environment, serving as a physical barrier and through the expression of key effector molecules of innate immunity, antimicrobial peptides (AMPs). There are two main AMP subfamilies, cathelicidins and defensins, which have broad-spectrum antimicrobial activity against many pathogen types, including bacteria, viruses and fungi. This study aimed to define the AMP expression profile of the equine cornea and conjunctiva as well as amnionic membrane, a tissue used widely as a bioscaffold for surgical repair of the cornea as it exhibits anti-inflammatory, antifibrotic, and antimicrobial properties. Thus, this study represents the initial characterization of the AMP repertoire of the equine amniotic membrane. Due to the gene sequence similarities in the beta-defensins and cathelicidins, orthologous genes were identified in the equine genome for investigation through a targeted qPCR approach. A preliminary interrogation identified expression of DEFB103A (beta-defensin 103A) and eCATH3 (equine cathelicidin 3) in corneal epithelium. DEFB103A expression was also identified in equine amniotic membrane. These preliminary data suggest that the equine corneal epithelium and amniotic membrane express AMPs, and further characterization with quantification will be performed to determine the expression of additional AMPs. This characterization of equine AMPs may provide important insights into the predisposition of horses to infectious keratitis, particularly fungal disease. Additionally, defining the AMP expression of the equine amnion can allow for prospective selection of amniotic membrane scaffolds with more potent AMP activity for the repair of significant corneal defects.

Impacts of the covid-19 pandemic on antimicrobial use in companion animals

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Judicious antimicrobial use (AMU) is important for preventing the evolution of antimicrobial resistance (AMR) in bacterial pathogens, which makes subsequent use of these drugs less effective in both human and veterinary medicine. Increased AMR prevalence and reduced development of novel antimicrobials has resulted in fewer treatment options for complex cases and multi-drug resistant (MDR) pathogens. Further, the Covid-19 pandemic required many practices to change their operations and decrease the number of patients seen, which may have been accentuated by changes in pet care seeking behaviors by owners. All of these changes may have impacted AMU during the pandemic. The goal of this research is to quantify changes in prescribing practices arising from the pandemic, as well as any potential downstream effects on AMR.

To do so, a retrospective study was performed using prescribing data collected from the pharmacy at NC State College of Veterinary Medicine's referral hospital and primary care centers for dogs and cats. This data contained records of all antimicrobial prescriptions from NC State from 2019-2020, and were used to categorize prescriptions as occurring before or during the pandemic. Each instance of AMU was counted to generate overall usage totals for each individual antimicrobial in the dataset. This study included 32,034 prescriptions, which were classified according to the FDA's system for ranking drugs of human medical importance. Using ordinal logistic regression, we calculated an odds ratio in R Studio to quantify the risk of each classification type being prescribed during the pandemic compared to beforehand. From this data, we seek to identify any potential changes in prescribing of antimicrobials as a result of the pandemic. Preliminary analysis found patients seen during the pandemic were significantly more likely to receive more important antimicrobials than before the pandemic (Crude OR = 1.1, $p < 0.005$).

Cell specific EphA4 alters immune cell recruitment and vascular remodeling following acute ischemic stroke

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Stroke is the fifth leading cause of death and a principle cause of long-term disability in the United States. The majority of stroke cases are ischemic in nature, resulting from a vascular obstruction that reduces cerebral blood flow and ultimately leads to permanent cell death and neurological deficits. Recent studies suggest pial collateral vessels, or leptomeningeal anastomoses, are a critical determinant of stroke outcome. Pial collateral vessels are formed during embryonic development and bridge distal arterioles in the pia mater of the brain. Under normal conditions, these vessels are small and inactive. Following ischemic stroke, these vessels remodel and enlarge through a process termed arteriogenesis, thereby allowing increased blood flow back into at-risk tissues. Using conditional endothelial cell (EC)-specific EphA4 knockout (*EphA4$^{fl/fl}$/Cdh5::Cre^{ERT2}*; KO) and wild type (*EphA4$^{fl/fl}$;* WT) mice, our findings suggest that the EphA4 receptor tyrosine kinase plays a major role in regulating pial collateral growth and remodeling after permanent middle cerebral artery occlusion (pMCAO). To analyze collateral size and cellular remodeling, we employed a novel method that combined vessel painting and immunohistochemistry techniques. We found that, compared to WT controls, KO mice displayed a significant reduction in infarct volume which correlated with larger ipsilateral pial collateral vessels as early as 4.5hrs and up to 24hrs post-pMCAO. In parallel to the increase in collateral size observed, a significant increase in the number of CD11b+ immune cells recruited to the collateral vessel was seen in KO mice at 6hrs post-pMCAO compared to WT controls. These findings demonstrate that EC-specific EphA4 limits collateral growth and size and is a novel therapeutic target for ischemic stroke treatment. Further time course analysis of cellular changes such as EC proliferation, smooth muscle cell reorganization and/or sub-type immune cell recruitment are being evaluated as contributors of the remodeling response.

Circumscribing laser cuts attenuate chronic focal cortical seizure propagation in mice

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Focal epilepsy, a subclass of epilepsy, is characterized by seizures that initiate in a small region of the brain and propagate out. Medical management fails in about 45% of these patients and resective surgery remains the only alternative, but often leaves patients with severe neurologic deficits. *In-vivo* two-photon imaging of acute seizure propagation has shown that seizures primarily propagate along lateral connections in cortical layers II-III. Therefore, it has been hypothesized that making minimally invasive incisions to severe lateral connections that seizures propagate along while maintaining vertical connections in the cortex would preserve much of normal brain function while blocking seizure propagation. Tissue ablation by tightly-focused femtosecond laser pulses provides a “laser scalpel” that can make subsurface microincisions in the cortex without damaging surrounding tissue. Here we tested the long-term efficacy of laser cuts in the supragranular layers of the neocortex in interfering with the propagation of seizures in a chronic model of focal seizures, and we examine the impact of the cuts on normal cortical structure and function. Chronic focal seizures were induced by microinjection of iron chloride. In mice with laser cuts in layers II-IV of the cortex that encircled the seizure focus, we observed an 80% reduction in seizure propagation over 3 months, as compared with controls. Chronic imaging of ablated regions with laser speckle contrast showed no significant reduction in blood flow to the encircled region and histology one month after ablation showed minimal inflammatory cells with a very small scar. When making these cuts in forelimb motor cortex we detected only a minor acute deficit when compared with sham animals in a complex reaching task. In conclusion, our results suggest cutting lateral connections in the supragranular cortical layers surrounding a seizure focus could be a promising neurosurgical approach that is efficient in blocking seizure propagation while maintaining most normal brain activity.

A biomaterial-based delivery system for immunotherapeutic cytokines

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Cytokines have important roles in cell-to-cell signaling pathways, including stimulating an anti-tumor immune response. Clinical utility of cytokine therapy is limited by short half life of the proteins and significant off-target effects. Development of a delivery system that could localize and prolong the activity of immune-activating cytokines might enhance the safety and efficacy of cytokine therapy as a cancer treatment. Our lab developed mineral-coated microparticles (MCMs) with a unique surface topography that binds proteins and sustains release over time. Here, we applied MCM technology to the delivery of interleukin 15 (IL-15), a pro-inflammatory cytokine with roles in T lymphocyte and natural killer cell activation.

MCMs were prepared by rotating core particles in simulated body fluid containing high amounts of calcium and phosphate. To bind cargo, MCMs were rotated in solutions containing varying concentrations of IL-15. Cytokine-loaded MCMs were either incubated in media to assess IL-15 release over time, or directly added to T cell culture to measure effects on cell proliferation. We demonstrated a prolonged release profile over at least two weeks in simulated body fluid. There was also notable T lymphocyte proliferation on day six after MCM-based delivery of IL-15 in culture. The ability of MCMs to bind and release bioactive IL-15 makes them a possible drug delivery option for future *in vivo* tumor studies. This could possibly mitigate current issues of rapid clearance and off-target side effects associated with cytokine therapy.

The novel innate immune-antagonistic effects of the multifunctional ectromelia virus C15 protein

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The success of poxviruses as pathogens depends upon their extensive antagonism of host immune responses by a large arsenal of immunomodulatory proteins. The C15 protein of ectromelia virus (ECTV, the agent of mousepox) is the largest of the ECTV immunomodulatory proteins and is a member of a well-conserved poxviral family previously studied as inhibitors of T cell activation. We have recently determined that C15 also facilitates viral spread in vivo by 3 days post infection, suggesting a second non-adaptive function of C15. Accordingly, we sought to further investigate this new function and identify the cellular target. We found this replication-promoting effect persists in the absence of T cells but is lessened in NK cell-deficient animals, implying the targeting of NK cells. Further investigation of NK cell function both *ex vivo* and *in vitro* shows that C15 selectively antagonizes degranulation of NK cells but not production of antiviral cytokines. Preliminary data suggests that the full impact of C15 *in vivo* is also reliant upon CD8 T cells, even at this early time point. These results prompt further investigation into the mechanism used by C15 to inhibit these cell types and demonstrate the discovery of a novel second function of the protein, which can selectively antagonize both the innate and adaptive murine immune responses.

Oxidant stress response in neonatal rats as a predisposing factor to lung developmental disruption by episodic ozone exposure

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Ozone is a powerful oxidant, and early life ozone exposure decreases lung function in humans. These functional changes are thought to be related to structural changes in the distal lung. The mechanism by which early life ozone exposure causes lung structural changes is unknown, but may be related to oxidant stress responses in the immature lung, which differ from those in the mature lung. In this study, we will identify mechanisms of ozone-induced change in the distal lung, investigating neonatal antioxidant capacity as a predisposing factor to airway remodeling. We will use an animal model of ozone-induced distal conducting airway growth restriction in the Sprague-Dawley rat to characterize levels of glutathione (GSH), an important cellular antioxidant, as well as expression and distribution of oxidant stress-related genes and protein products such as glutathione-s-transferase and Club cell secretory protein. We hypothesize that oxidant stress responses to ozone in the distal neonatal lung differ from those in mature lungs. Specifically, we hypothesize that the distal neonatal lung is less able than the mature lung to upregulate cellular antioxidant responses to ozone. Decreased GSH re-synthesis and antioxidant enzyme expression and altered distribution in the distal lung would implicate immature neonatal antioxidant responses as predisposing factors to ozone-induced structural changes. This attenuated antioxidant response may predispose neonates to disrupted lung development due to ozone exposure. Together with studies on structural changes induced by ozone, these studies will help us identify the mechanism by which early life ozone exposure alters normal lung development, a neglected area of research.

A Uropathogenic E. coli UTI89 model of prostatic inflammation and collagen accumulation for use in studying aberrant collagen production in the prostate

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Bacterial infection is one known etiology of prostatic inflammation. Prostatic inflammation is associated with prostatic collagen accumulation and both are linked to progressive lower urinary tract symptoms in men. We characterized a model of prostatic inflammation utilizing transurethral instillations of E. coli UTI89 in C57BL/6J male mice with the goal of determining the optimal instillation conditions, understanding the impact of instillation conditions on urinary physiology, and identifying ideal prostatic lobes and collagen 1a1 prostatic cell types for further analysis. The smallest instillation volume tested (50 µL) distributes exclusively to bladder, 100 and 200 µL volumes distributes to bladder and prostate, and a 500 µL volume distributes to bladder, prostate and ureter. A threshold optical density (OD) of 0.4 E. coli UTI89 in the instillation fluid is necessary for significant ($p < 0.05$) prostate colonization. E. coli UTI89 infection results in a low frequency, high volume spontaneous voiding pattern. This phenotype is due to exposure to E. coli UTI89, not catheterization alone, and is minimally altered by a 50 µL increase in instillation volume and doubling of E. coli concentration. Prostate inflammation is isolated to the dorsal prostate and is accompanied by increased collagen density. This is partnered with increased density of PTPRC+, ProCOL1A1+ co-positive cells and decreased density of ACTA2+, ProCOL1A1+ co-positive cells. Overall, we determined that this model is effective in altering urinary phenotype and producing prostatic inflammation and collagen accumulation in mice.

Ubiquitin ligase SMURF2 interacts with filovirus VP40 and promotes egress of VP40 VLPs

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Filoviruses Ebola (EBOV) and Marburg (MARV) are devastating high-priority pathogens capable of causing explosive outbreaks with high mortality rates. The matrix proteins of EBOV and MARV, called eVP40 and mVP40, respectively, are the key viral proteins that drive virus assembly and egress and VP40 can bud independently from cells in the form of virus-like particles (VLPs). The matrix proteins utilize proline-rich Late (L) domain motifs (e.g., PPxY) to hijack specific host proteins that contain WW domains, such as the HECT family E3 ligases, to facilitate the last step of virus-cell separation. We identified E3 ubiquitin ligase Smad Ubiquitin Regulatory Factor 2 (SMURF2) as a novel interactor with VP40 that positively regulates VP40 VLP release. Our results show that eVP40 and mVP40 interact with the three WW domains of SMURF2 via their PPxY motifs. We provide evidence that the eVP40-SMURF2 interaction is functional as the expression of SMURF2 positively regulates VLP egress, while siRNA knockdown of endogenous SMURF2 decreases VLP budding compared to controls. In sum, our identification of novel interactor SMURF2 adds to the growing list of key host proteins that can regulate PPxY-mediated egress of VP40 VLPs. A more comprehensive understanding of the modular interplay between filovirus VP40 and host proteins may lead to the development of new therapies to combat these deadly infections.

Enhancing ADCC by natural killer cells to improve ovarian cancer tumor cell killing

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Ovarian cancer is the most lethal gynecologic malignancy. Despite radical surgical and adjuvant therapies, many ovarian cancer patients develop therapeutic resistance. Natural killer (NK) cells are innate cytotoxic lymphocytes that kill cancer cells without prior sensitization. A key NK cell function is antibody-dependent cell-mediated cytotoxicity (ADCC), and many clinically successful therapeutic monoclonal antibodies (mAbs) utilize ADCC to rapidly kill antibody-opsonized tumor cells. To date, NK immunotherapies have not been thoroughly investigated for treatment of ovarian cancer. CD16A, an IgG Fcγ receptor (FcγR), is the sole means of human NK cell recognition for anti-tumor antibodies. However, CD16A is low affinity and is cleaved by ADAM17 upon activation, potentially reducing the efficacy of mAbs. CD64, which is expressed on myeloid cells, is the highest affinity FcγR and is not cleaved by ADAM17. We hypothesize that engineering NK cells to express recombinant CD64 will improve ADCC of ovarian cancer. We generated CD64/16, a novel high-affinity recombinant FcγR, that consists of the extracellular region of CD64 and transmembrane and intracellular regions of CD16A. We expressed CD64/16A in human NK-92 cells and induced pluripotent-derived stem cells that were differentiated into NK cells. We found that CD64/16 facilitated intercellular conjugation between tumor and NK cells, produced cytokines, and killed ovarian cancer via ADCC. Using a mouse xenograft model, we show that CD64/16 NK cells reduce tumor burden. Finally, we observed that CD64/16 can serve as a universal anti-tumor antibody docking platform while retaining ADCC efficacy. Our findings provide new insights into using Fc receptor-based NK cell therapies and lay the preclinical foundation for development of an “off-the-shelf” cellular therapy that can be combined with therapeutic tumor-targeting mAbs for the treatment of not only ovarian cancer, but other malignancies. Furthermore, our data may provide an opportunity for “reverse translation” for NK cell-based cancer immunotherapies for companion animals.

Applying chain graph models to identify livestock management risk factors for antimicrobial resistance among *Campylobacter coli* from agricultural swine populations

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Antimicrobial resistance (AMR) is a major threat to human and animal health today, rendering once treatable infections untreatable. Although antimicrobial use is known to directly select bacterial populations that are resistant to those drugs, other important risk factors influencing the epidemiology of AMR are incompletely understood. Several complex mechanisms enable the exchange of genetic material among populations of unrelated bacteria (e.g. mobile genetic elements), thereby influencing the rise of multidrug resistant bacterial populations. Thus, resistance to an antibiotic may still arise even without using that specific antibiotic. Traditional analytical methods used in epidemiological research rely solely on generalized linear models to identify risk factors for health outcomes; however, these methods are less suitable for evaluating AMR risk factors while simultaneously accounting for the complex, non-linear dynamics of AMR selection and persistence. In this study, we utilize a multi-layered chain graph model to identify risk factors for phenotypic resistance while also accounting for potential complex genetic mechanisms underlying AMR selection and persistence. We applied this model to populations of *Campylobacter coli* isolated from agricultural swine herds experiencing varying degrees of antimicrobial exposure and other management practices. In addition to antimicrobial usage, we found that risk for fluoroquinolone- and macrolide-resistance differed based on biosecurity practices employed at each farm and whether animals were reared entirely indoors or outdoors. Results and computational methods from this study are applicable to human public health surveillance data.

Multispecies proteomics reveals potential biomarkers for osteoarthritis

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Post-traumatic osteoarthritis (PTOA) following osteochondral fragmentation in horses, cranial cruciate ligament rupture (RCCL) in dogs, and anterior cruciate ligament (ACL) injury in humans is a leading cause of disability across all three species. The properties of RCCL in dogs has made it an attractive target as a comparative animal model for ACL injury in humans, while PTOA in horses has comparative relevance to human intra-articular fracture. Prior proteomics research has identified possible biomarkers for osteoarthritis in dogs and humans. The goal of this study was to reveal protein biomarkers and potential therapeutic targets for osteoarthritis (OA) in synovial fluid across the three species.

Synovial fluid samples were collected from 16 equine carpal joints (n=8 PTOA, n=8 healthy), 16 canine stifle joints (n=8 RCCL, n=8 healthy), and 10 human knee joints (n=10 injured limb, n=6 uninjured limb). Human samples were paired, while dog and horse samples were obtained from independent animals. Proteomics data was collected using nano-scale reverse phase chromatography and tandem MS (nanoLC-MS/MS), followed by identification using Proteome Discoverer 2.3 and analysis of proteins in MetaboAnalyst 5.0.

In all species, Principal Component Analysis (PCA) demonstrated distinct groupings between healthy and OA samples (**Figure 1**). Several proteins were significantly up- or down-regulated in OA compared to healthy synovial fluid samples across two or more species, and five proteins were upregulated in OA samples in all species (**Figure 2**). A multi-species approach enables more robust identification of proteins of interest and may offer insight into naturally occurring animal models for human OA. Future work may use the results of this study to focus therapeutic or OA screening protocol development.

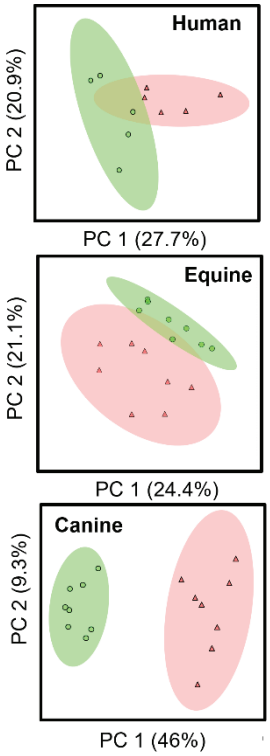


Figure SEQ Figure * ARABIC 1. PCA of synovial fluid proteomics. Red markers indicate OA samples, while green markers indicate healthy samples

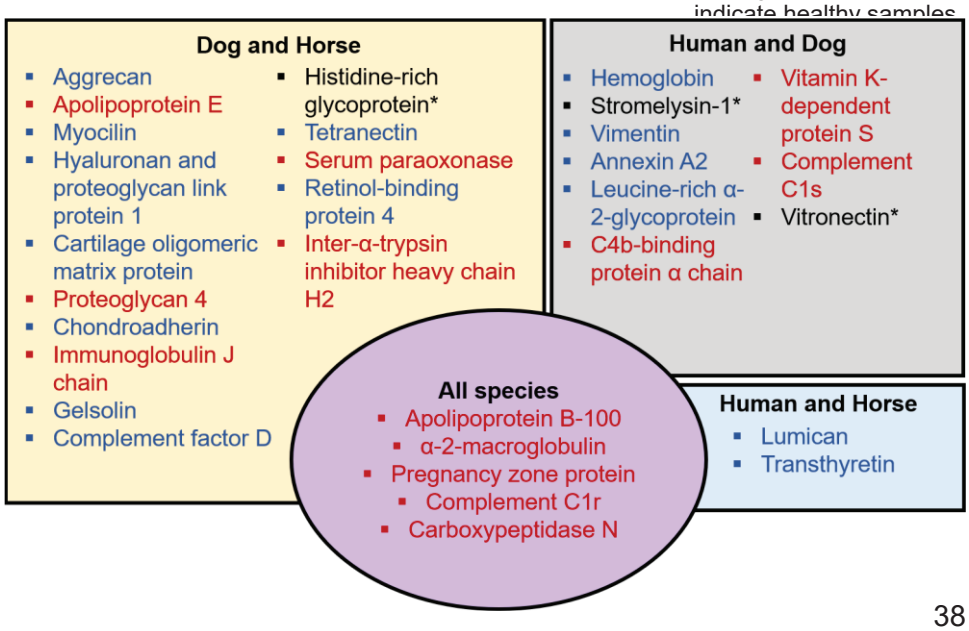
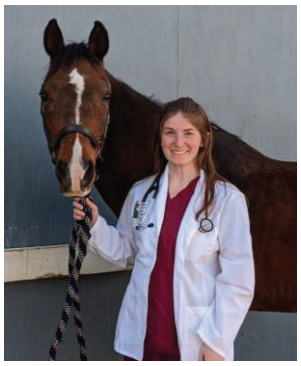


Figure 2. Proteins organized by regulation among species. Proteins listed are different between OA and healthy groups with at least 90% confidence (not FDR-corrected) and are among the top 50 proteins ranked by fold-change per species. Proteins in red are upregulated in OA, proteins in blue are downregulated in OA, and proteins with asterisks exhibit species-dependent regulation.

Colloquium Planning Team



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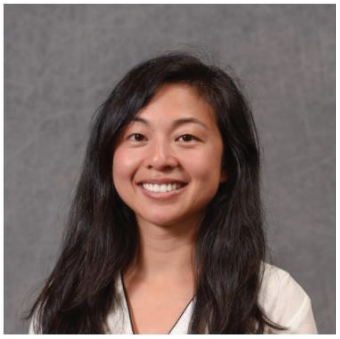

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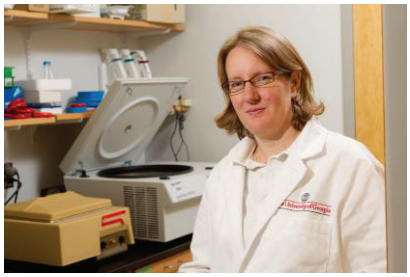

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