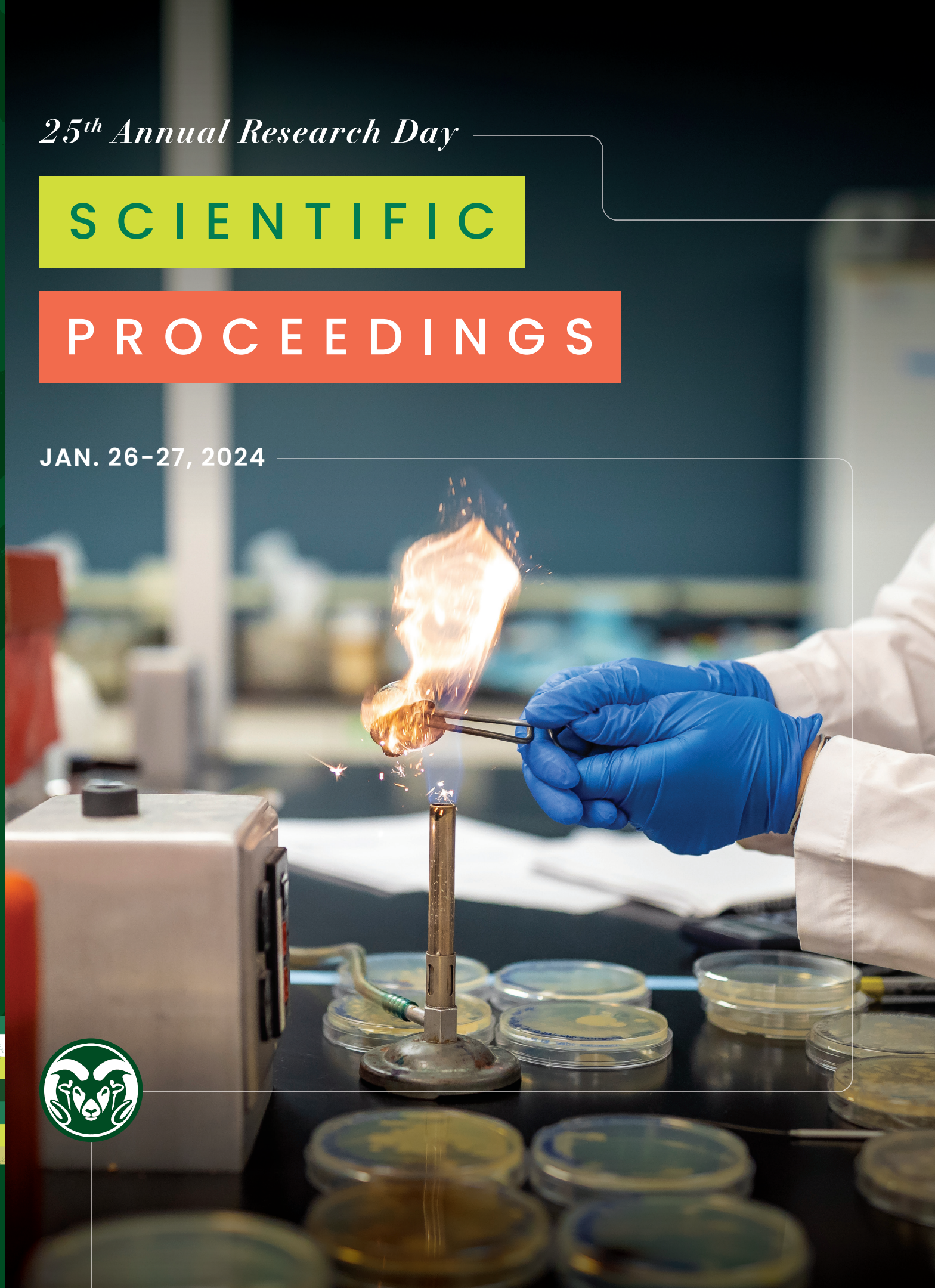
25th Annual Research Day

SCIENTIFIC PROCEEDINGS

JAN. 26–27, 2024



Our 25th annual Research Day showcases the work of nearly 200 aspiring scientists in Colorado State University's College of Veterinary Medicine and Biomedical Sciences. The event gives our rising stars vital experience presenting their research findings to a scientific audience through poster displays and talks. The day also provides young researchers with an avenue for feedback to help them develop ideas that, in many cases, will become lifelong scientific pursuits.

The research projects on display are sponsored by companies, foundations, and institutions concerned with improving human, animal, and environmental well-being. Thank you for supporting and engaging with our presenters – undergraduate students, graduate students, veterinary residents, and post doctoral fellows – as they pursue research that will improve the health of animals, people, and the planet!

THANK YOU TO OUR SPONSORS!

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2024 CVMBS Research Day Organizing Committee

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Faculty Co-Chair – Microbiology, Immunology, and Pathology

Katie Sikes
Faculty Co-Chair – Clinical Sciences

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Faculty Co-Chair – Microbiology, Immunology, and Pathology

Aimee Oke
Committee Coordinator – CVMBS Dean's Office

Vanessa Selwyn
Committee Coordinator – CVMBS Dean's Office

Wendy Stevenson
Committee Coordinator – CVMBS Dean's Office

CONTENTS

RESEARCH DAY 2024

SCHEDULE OF EVENTS

Friday, January 26 - Translational Medicine Institute

6 p.m.	Doors Open	TMI Lecture Hall
6:30-7:30 p.m.	Distinguished Lecture, Dr. Cheryl London	TMI Lecture Hall
7:30-8:30 p.m.	Zoetis Research Awardee Reception	TMI Grand Hall

Saturday, January 27 - Lory Student Center

9-10 a.m.	Check-in and Poster Setup	LSC Upper Lobby
10-11 a.m.	Zoetis Research Excellence Recipient Keynote: Dr. Lynn Pezzanite	LSC Theatre
11 a.m.-noon	Undergraduate Poster Session	LSC Theatre
12:15-4 p.m.	Podium Presentations: Basic Science	RM 1: LSC 308-310
	Podium Presentations: Basic Science	Rm 2: LSC 312
	Podium Presentations: Clinical Science	Rm 3: LSC 322
	Podium Presentations: Translational Science	Rm 4: LSC 302
	Poster Sessions	LSC Grand Ballroom A/B
4:15-6:30 p.m.	Social Hour and Awards	LSC Grand Ballroom C/D

DEPARTMENTAL ABBREVIATIONS

BMS: Biomedical Sciences
CS: Clinical Sciences
ERHS: Environmental and Radiological Health Sciences
MIP: Microbiology, Immunology, and Pathology

CONGRATULATIONS TO THE 2023 CVMBS RESEARCH DAY WINNERS!

ORAL PRESENTATIONS

- **EARLY-STAGE BASIC RESEARCH - 1ST PLACE** Molly Ring (Brian Foy, MIP): Localization of a probiotic Lactobacillus acidophilus rotavirus vaccine within the host

- **EARLY-STAGE BASIC RESEARCH - 2ND PLACE** McKenzie Fletcher (Gregg Dean, MIP): The Role of Niemann Pick Type C2 Genes During Ivermectin Blood Meal Response in Mosquito Plasmodium Vectors

- **ADVANCED-STAGE BASIC RESEARCH - 1ST PLACE** Samantha Labb (Ralf Sudowe, ERHS): Development of a sodium bismuthate-coated polyacrylonitrile resin for the separation of oxidized actinides from used nuclear fuel

- **ADVANCED-STAGE BASIC RESEARCH - 2ND PLACE** Jessica Kincade (Thomas Hansen, BMS): Fetal BVDV infections and postnatal epigenetic dysregulation

- **EARLY-STAGE CLINICAL RESEARCH - 1ST PLACE** Katya Spitznagel (Michala de Linde Henriksen, CS): Detection of pro-inflammatory cytokines in healthy canine tear film using mass spectrometry via multiple reaction monitoring (MRM-MS)

- **EARLY-STAGE CLINICAL RESEARCH - 2ND PLACE** Nina Marchell (Elissa Randall, ERHS): Radiation dose to patients and staff during feline esophagrams

- **ADVANCED-STAGE CLINICAL RESEARCH - 1ST PLACE** Devon Diaz (Julien Guillaumin, CS): Impact of lyophilized platelet infusions on point-of-care viscoelastic test VCM-Vet™ in canine cardiopulmonary bypass

- **EARLY-STAGE TRANSLATIONAL RESEARCH - 1ST PLACE** Emily Van Zeeland (Katie Sikes, CS): Sex differences in the response to anterior cruciate ligament injury following mechanical rupture in a mouse model

- **EARLY-STAGE TRANSLATIONAL RESEARCH - 2ND PLACE** Marika Klosowski (Dan Regan, MIP): Breast cancer cell co-culture with primary lung fibroblasts is associated with modulation of cytokine secretion and extrinsic stromal-mediated drug resistance

CONGRATULATIONS TO THE 2023 CVMBS RESEARCH DAY WINNERS!

POSTER PRESENTATIONS

- **EARLY-STAGE BASIC RESEARCH - 1ST PLACE** Brody Thomas (Traci Kinkel, MIP): The Effect of Water Bowl Material and Volume on Downstream Biofilm Formation in Canines

- **EARLY-STAGE BASIC RESEARCH - 2ND PLACE** Leo Tyer (Glenn Telling, MIP): Exploratory use of RT-QuIC to quantify and compare kinetics of amyloid formation in North American and Scandinavian CWD prions

- **ADVANCED-STAGE BASIC RESEARCH - 1ST PLACE** Elena Lian (Mary Jackson, MIP): The functional impact of ubiA mutations associated with chronic Mycobacterium abscessus infections – implications for host adaptation

- **EARLY-STAGE CLINICAL RESEARCH - 1ST PLACE** McKenzie Sparrer (Candace Mathiason, MIP): Surveillance of companion and exotic animals for SARS-CoV-2 and evaluating transmission potential within veterinary medicine

- **EARLY-STAGE CLINICAL RESEARCH - 2ND PLACE** Thomas Burnett (Anne Avery, MIP): Quantitative assessment of genes involved in early lymphopoiesis using droplet digital PCR (ddPCR) to distinguish immature lymphoid neoplasms in the dog

- **EARLY-STAGE TRANSLATIONAL RESEARCH - 1ST PLACE** Alice (Neely) Droeger (Julie Moreno, ERHS): Glial inflammation in aging canines

- **EARLY-STAGE TRANSLATIONAL RESEARCH - 2ND PLACE** Caroline Kern-Allely (Colleen Duncan, MIP): Waste not want not: Piloting a clinical waste audit at a university veterinary teaching hospital

- **ADVANCED-STAGE TRANSLATIONAL RESEARCH - 1ST PLACE** Hayley Templeton (Stuart Tobet, BMS): Intestinal Neuroimmune Involvement in Parkinson's Disease Development

GOLDEN PIPETTE AWARD

MICROBIOLOGY, IMMUNOLOGY AND PATHOLOGY

DR. LYNN PEZZANITE EXPANDS ON KEY ORTHOPEDIC RESEARCH IN HORSES THAT COULD ALSO AID HUMANS

By Chris Outcalt

It was early November, and Dr. Lynn Pezzanite was standing in an operating room at Colorado State University's Translational Medicine Institute, flanked on either side by two of her most influential mentors, Dr. Laurie Goodrich and Dr. Jason Stoneback. Goodrich and Stoneback had scrubbed in to assist on a pair of equine surgeries tied to Pezzanite's first research grant as the principal investigator.

"They've been such supporters collectively in my success," Pezzanite said. "It was surreal to be in that position."

Building on findings from her post-doctoral work, Pezzanite, an equine surgeon and an assistant professor at CSU's Veterinary Teaching Hospital, was embarking on a study designed to better understand how immune cells behave as osteoarthritis progresses in horses. The degenerative joint disease is one of the most common disorders that equine veterinarians encounter, impacting up to 80% of horses older than 15. Treatment options, however, remain limited.

The idea behind Pezzanite's study — funded by a two-year grant from the nonprofit Grayson-Jockey Club Research Foundation — was that examining the early-stage immune response to osteoarthritis might allow the group to identify certain biological markers

of different stages of the disease. From there, the team could perhaps tailor treatments or gene therapies to specific stages, helping prevent the disease's progression. "We're really trying to look at: What is the best timing to intervene?" Pezzanite said.

HELPING HORSES AND HUMANS

Stoneback's presence in the operating room signaled another research goal. As the University of Colorado Anschutz Medical Center chief orthopedic trauma surgeon and director of CU's Limb Restoration Program, Stoneback works with humans, not horses. Nevertheless, he views the research being done by Pezzanite, Goodrich – who directs CSU's Orthopaedic Research Center – and others at the Translational Medicine Institute, including Dr. Steven Dow, as potentially "game-changing" for his own practice.

"The work going on here," Stoneback said, "could have direct translational impact in humans."

"I INITIALLY VIEWED THE POTENTIAL FOR OUR WORK TO HELP PEOPLE AS AN ADDED BENEFIT, AND NOW, I HONESTLY VIEW WORK THAT BENEFITS BOTH ANIMALS AND PEOPLE AS OUR PRIMARY FOCUS."

Stoneback is among a small group of physicians worldwide who regularly perform an innovative procedure that involves implanting a metal orthopedic rod into an amputee's residual limb. The method, known as osseointegration or bone-anchored limb replacement, is considered superior to fitting an amputee with a traditional prosthesis because the implant connects directly with the patient's body, helping to improve awareness, balance and gait.

A world-class human surgeon, Stoneback had been looking forward to the experience in the equine operating room. "Instead of being the person who people are looking to for guidance and directing exactly what's going to happen, I'm here as an observer and a participant," he said. "It's super cool to come and learn from such exceptional people."

During surgery, the team worked to create a tiny chip in the horse's cartilage. The procedure is designed to induce a small amount of inflammation in the joint, prompting an immune response. During the next four months, Pezzanite would then monitor and study what happened. "I'll take tissue samples and look at how the gene expression changes at a single cell level," Pezzanite said. "Hopefully that will inform us further on biomarkers of disease progression and the best timing to intervene."

CSU A HUB FOR ORTHOPEDIC RESEARCH

Pezzanite attended vet school in upstate New York at Cornell University. Her mother was a veterinarian, and Pezzanite always had a sense that she would spend her career working with horses too. When it came time to apply for residency, Pezzanite was attracted to CSU because of the University's strong reputation as a hub for orthopedic research. "This was my top choice," Pezzanite said. "I really only wanted to come here."

After residency, Pezzanite remained at CSU to work on her post-doctoral degree. She dug further into the intersection of orthopedic research and immunology, collaborating with Goodrich and Dr. Steven Dow, director of CSU's Immunotherapy Research Laboratory housed within the Translational Medicine Institute and Flint Animal Cancer Center.

Working in vitro as well as in rodent models, Dow had pioneered immunotherapies designed to treat antimicrobial-resistant infection. That work presented an opportunity for Pezzanite: Testing the effectiveness of Dow's therapies in horses became the foundation of her post-doc research. "I was starting my Ph.D.," Pezzanite said, "and we were interested in taking this forward in a larger animal model."

Around the same time, Dow met CU's Stoneback at a conference in Denver. When Stoneback learned about Dow's immunotherapy research an idea clicked: He thought the immune-activated cellular therapies might help with the infection challenges he sees with his patients and expressed interest in integrating the

work into his limb restoration practice. From there, a wider partnership formed. Stoneback joined Pezzanite's post-doctoral committee, and she regularly traveled to Denver to shadow Stoneback in his operating room and learn from his practice.

In October 2022, Pezzanite, Dow and Goodrich published promising results from the horse therapy trials in the journal Annals of Translational Medicine. After that, Pezzanite began to think through next steps, both for the research and for her career. She had multiple offers to join faculty at other schools but decided to stay in Fort Collins in no small part because of the support from Dow, Goodrich and Stoneback. "All three of them have been instrumental in me staying," she said.

The experience with Stoneback's practice in particular made Pezzanite realize that she wanted to focus specifically on translational medicine, researching therapies that would benefit animals but that could also be applied to advancements in human medicine.

"I initially viewed the potential for our work to help people as an added benefit," Pezzanite said. "And now, I honestly view work that benefits both animals and people as our primary focus."

AN EXCELLENT TRANSLATIONAL SCIENTIST

Within the past 10 years, Goodrich said, the concept of translational medicine has become more widely accepted and practiced in the broader scientific research community. "Right now, across the country and the world, you're seeing a lot of translational medicine centers pop up," Goodrich said. "But very few of them are at veterinary institutions."

Pezzanite, Goodrich said, is an excellent example of exactly that kind of translational scientist. "She's been outstanding in every part of her training here," Goodrich said. "She has an amazing capacity to take an idea and turn it into a grant and execute the work — and she's a great mentor to her students as well."

Pezzanite plans to have some initial results from her research grant sometime this summer. From there, she hopes to expand her work, both building on the osteoarthritis study and pursuing other ways to treat infections in patients with orthopedic implants. "Musculoskeletal, particularly joint-related, disease is a major focus for my program moving forward," she said. "We look forward to continued collaborations with Dr. Stoneback and his team aimed at improving outcomes in post-traumatic orthopedic conditions. The goal is to eventually run a clinical trial in humans."

Even then, Pezzanite might still be reflecting on how much that day in the operating room with Goodrich and Stoneback meant to her and her career.

UNDERGRADUATE POSTER PRESENTATIONS

11 A.M. - NOON | LSC THEATRE

No.	Presenter	Title | Mentor	Dept.
1	Anderson, Isla	Peripheral *Mycobacterium tuberculosis* Infection Results in Neuroinflammation and accumulation of Misfolded Proteins in Non-Human Primates | A Latham	ERHS
2	Aragon, Victoria	Haploinsufficiency of the autism-associated δ-catenin mutation is sufficient to induce social dysfunction in mice | S Kim	BMS
3	Bennett, Lauren	Evaluating the presence of Chronic Wasting Disease in free-range White-tailed deer in Arkansas | N Denkers	MIP
4	Black, Ellison	Selective cholinergic activation prevents memory loss and the *in vivo* growth of amyloid plaques in Alzheimer's disease | S Kim	BMS
5	Bouckova, Evelina	Ketamine reverses chronic stress-induced mental disorders via the expression of Ca2+-permeable AMPA receptors in mice | S Kim	BMS
6	Bovaird, Emma	An exploration of the stability and integrity of ready-to-use therapeutic foods in Indonesia | E Ryan	ERHS
7	Byfield, Isabelle	Exploring CYP6AG7's role in pesticide resistance in *Aedes aegypti* | K Saavedra Rodriguez	MIP
8	Conner, Daryl	Factors influencing the pathogenesis of Tuberculosis meningitis in mice | M Henao Tamayo	MIP
9	DeBie, Ashley	Unraveling the IL-24 Molecular Mechanisms of Dust-Induced Lung Inflammation in Mice | T Nordgren	ERHS
10	Dirks, Kaitlyn	Antiviral roles of sphingolipid metabolism | R Perera	MIP
11	Flatt, Tatum	Neuropathological Changes in the Aged Canine with Canine Cognitive Dysfunction | J Moreno	ERHS
12	Galvan, Michelle	Investigating SARS-CoV-2 evolution and transmission in lions, tigers, and hyenas at the Denver zoo using low-copy late-stage nasal swabs | S VandeWoude	MIP
13	Hatch, Nizhoni	Identifying Plasma Biomarkers for ALS Utilizing Human and SOD1-G93A Mouse Model | P Charley	MIP

No.	Presenter	Title | Mentor	Dept.
14	Hebert, Emma	Investigation of health education resources for students at Colorado State University regarding regional vector-borne disease | S Magzamen	ERHS
15	Hopkins, Caleb	Characterization of the locomotor changes and neuroinflammatory phenotypes in Gerstmann-Straussler Scheinker (GSS), a familial prion disease | J Moreno	ERHS
16	Jacobs, Kyla	NPY intracranial ventricular injection suppresses LH pulsatile secretion in adult female mice | R McCosh	BMS
17	Jones, Maya	Dietary inclusion of rice bran increases functional food properties of meals and snacks | E Ryan	ERHS
18	Judson, Madeline	Testing the aerosolization of bacteria from an inoculating loop | D Hyatt	MIP
19	Jung, Marcie	Extracellular Vesicles from Oviductal Organoids Capacitate Bull Sperm Enabling Fertilization In Vitro | J Graham	BMS
20	Korte, Colin	Modulation of *Culicoides sonorensis* Microbiome in Response to Veterinary Pharmaceuticals | G Borlee	MIP
21	Martinez, Olivia	Investigating the Effects of Temperature Change on Oviposition and Progeny Viability of *Aedes aegypti* and *Culex tarsalis* Mosquitoes | R Kading	MIP
22	McGrail, Dayton	Extraction of photolumazine with MR1-restricted T cell activity from an environmental bacterium isolated from the soil | K Dobos	MIP
23	Moore, Katie	The use of glycopeptidolipids as a diagnostic biomarker to detect non-tuberculosis infections in Cystic fibrosis patients. | D Chatterjee	MIP
24	Mumford, Genova	An in-vivo exploration of the impact of the IKK Kinase complex on prion-induced inflammation | J Moreno	ERHS
25	Namesnik, Luke	Susceptibility of Jamaican fruit bats wing punch cells to Cedar virus | P Charley	MIP
26	Pauly, Morgan	Inhaled agricultural dust exposure protects against secondary Streptococcus pneumoniae infection. | T Nordgren	ERHS
27	Perkins, Emily	Establishing molecular mechanisms of glial-mediated response in a chronic pain *in vitro* model | K Popichak	MIP

No.	Presenter	Title | Mentor	Dept.
28	Pogge, Quinn	Glial inflammatory response to metronidazole exposure in a murine, in vitro model | K Popichak	MIP
29	Prosceno, Isabella	Cheating death by gambling with chromosomes: karyotypic variation drives the emergence of virulent traits in an opportunistic fungal pathogen | L Heasley	BMS
30	Scarsbrook, Matthew	Tibialis Anterior Maximal Force Capacity and Balance Assessment Scores in People with Multiple Sclerosis | B Fling	HES
31	Smith, Emma	An integrated analysis of the transcriptomic effects observed following wildland firefighter relevant occupational exposures in the pre-frontal cortex of male mice | L Montrose	ERHS
32	Wahl, Maelis	Characterization of Gene Expression in Increased Omega-3 Fatty Acid Model Following Organic Dust Exposure | T Nordgren	ERHS
33	Weisman, Grace	Prevention of inflammation and loss of myelin in a mouse model of Multiple Sclerosis using Nanoligomers™ | J Moreno	ERHS
34	Weninger, Kristin	Neuropathological brain changes in a Dunkin-Hartley Guinea Pig Model as a naturally occurring model of Alzheimer's Disease | J Moreno	ERHS
35	Wienke, Mackenzie	Characterization of modes and kinetics in mutation accumulation models in *Saccharomyces cerevisiae* | JL Argueso	ERHS
36	Wiles, Mckennon	Ketamine's rapid antidepressant effects are mediated by Ca2+-permeable AMPA receptors | S Kim	BMS

SESSION 1: Basic Science

12:15–3:45 p.m. | LSC 308-310

Time	Presenter	Topic	Dept.
12:15	Bahadur, Sami	Is the Occurrence of Antimicrobial Resistance Genes Higher on Chromosomes Compared to Plasmids among *Salmonella Typhimurium* Isolates from Cattle? | S Rao	CS
12:30	Boxleitner, Taryn	Immunomodulatory effects of articular chondroprogenitor cells | L Goodrich	CS
12:45	Bradley, Matthew	Behavioral strategies of state animal health officials on national disease reporting | S Rao	CS
1:00	Cole, Jesse	Extracellular vesicles as a potential mechanism of hematogenous transport of Chronic Wasting Disease | C Mathiason	MIP
1:15	Dunham, Tillie	Optimization of bluetongue virus RNA extraction and next-generation sequencing protocols for enhancement of disease surveillance | C Mayo	MIP
1:30	Forrest, Kaitlyn	Characterizing the Circadian Rhythms of PrP-Associated Transgenic Mice | C Mathiason	MIP
1:45	Grabowski, Christian	Health Effects of Uranium Contamination In Water On The Sweetwater Chapter Of The Navajo Nation | T Johnson	ERHS
2:00	BREAK		
2:15	Ramadan, Amr	Characterization of the surface-associated polysaccharides in *Burkholderia pseudomallei* | B Borlee	MIP
2:30	Ramirez, Ghyslaine	Enhancing the antioxidant capacity of granulosa cells to mitigate the impact of heat stress | D Tesfaye	BMS
2:45	Sewor, Christian	Household air pollution and Inflammation: do lifestyle patterns matter? | M Clark	ERHS
3:00	Thomas, Marshall	Spatial variation in access to alcohol, tobacco, and marijuana and association with sociodemographic characteristics in Denver, Colorado | S Magzamen	ERHS
3:15	Torres, Sophia	Tertiary lymphoid structures in canine soft tissue sarcomas: characterization and effect on prognosis | C Olver	MIP
3:30	Treuting, Quinn	Designing and implementing human factor simulation for assessing optimal PPE behaviors | S Rao	CS

SESSION 2: BASIC SCIENCE

12:15–4:00 p.m. | LSC 312

Time	Presenter	Topic	Dept.
12:15	Cameron, Kimona	Characteristics of the canine and feline trauma patients served by emergency and critical care services pre- and post the COVID-19 pandemic in Colorado | S Rao	CS
12:30	Dearing, Carley	Heart of the matter: sex-specific cardiac remodeling in aged rats exposed to early-life chronic stress is predicted by glucocorticoid responsivity | B Myers	BMS
12:45	Dickson, Ariana	Preferred treatment choices for trauma patients to improve survival outcomes: a pilot study | S Rao	CS
1:00	Holec , Sara	Effect of neuroinvasion on strain property maintenance for two α-synuclein prion strains | A Woerman	MIP
1:15	Kim, Elizabeth	Evaluating dairy farming interests of K-12 and college students utilizing virtual reality as an educational tool | S Rao	CS
1:30	Kincade, Jess	A tail of two calves: an epigenetic timeline of BVDV infections | T Hansen	BMS
1:45	Lowe, Diana	Adaptation of non-lymphotropic emergent Nordic CWD to a lymphotropic strain | G Telling	MIP
2:00	BREAK		
2:15	Lunsford, Elizabeth	Denver's tree canopy coverage: perspectives from the Globeville, Elyria, Swansea community health study | S Magzamen	ERHS
2:30	Maichak, Courtney	West Nile Virus Mosquito Vector Changes and Land Use Changes in Fort Collins, Colorado, 2006-2021 | S Magzamen	ERHS
2:45	Marano, Jeffrey	Pathogenesis and transmission of severe fever with thrombocytopenia syndrome virus in experimentally infected animals | A Bosco-Lauth	BMS
3:00	Oketade, Nurudeen	MR-1 antigenicity is dependent on riboflavin biosynthesis in *Mycobacterium tuberculosis* | K Dobos	MIP
3:15	Ratnayake, Oshani	Metabolic makeover: Investigating the role of metabolic remodeling in the mosquito vector during arbovirus infections | R Perera	MIP
3:30	Walker, Audrey	Susceptibility of ectotherms and house sparrows to Japanese encephalitis virus (JEV) genotypes I-IV | R. Bown and A. Bosco-Lauth	BMS

SESSION 3: CLINICAL SCIENCE

12:15–4:00 p.m. | LSC 322

Time	Presenter	Topic	Dept.
12:15	Baird, Taylor	Evaluation of viscoelastic testing and coagulation/hemostasis data in a canine hemorrhagic shock/resuscitation model | J Guillaumin	CS
12:30	Talbot, Charlie	Arterial blood gas parameters in healty, acclimated non-brachucephalic dogs at 1,535 altitude | K Zersen	CS
12:45	Neal, Katherine	Prevalence and association of pancreatitis in dogs with hypercalcemia | A Marolf	ERHS
1:00	Paulos, Trish	The use of doxorubicin and propranolol for canine splenic hemangiosarcoma: a retrospective study of 31 dogs | D Thamm	CS
1:15	Ray, Christopher	Ancillary treatments with thrombolytics or medications aimed at improving functional recovery or both in acute feline aortic thromboembolism | J Guillaumin	CS
1:30	Ryan, Mark	Evaluating the efficacy of shelf-stable blood products for resuscitation in a canine hemorrhagic shock model; an endothelial glycocalyx evaluation | K Hall	CS
1:45	Stadler, Megan	First Day Readiness-Basic Radiographic Clinical Interpretation-4th year Veterinary Students | L Griffin	CS
2:00	BREAK		
2:15	Barnes, Summer	Anesthesia of Madagascar Hissing Cockroaches (*Gromphadorhina portentosa*) with Isoflurane, and a Novel Technique for Heart Rate Monitoring | K Mama, M Sadar	CS
2:30	Cerna, Petra	Preliminary data on evaluation of new immunotherapy for cats with FIP being treated with EEID-2801 antiviral | M Lappin	CS
2:45	Felker, Jennifer	Improving health care literacy among patients with multiple sclerosis through a flipped classroom model | A Miravelle, UC Anschutz	CS
3:00	Gualtieri, Patricia	Stereotactic body radiation therapy for oral tumors in canine patients | K Boss	CS
3:15	Stromberg, Stephanie	Radiographic appearance of the cecum in dogs | E Randall	ERHS
3:30	Tucker, Claire	Evaluating the confidence and competency of leadership, teamwork, and communication skills in veterinary students during the RECOVER CPR training course | K Hall	CS
3:45	Weber, Annika	Solutions to Enhance Health with Alternative Treatments (SEHAT): a double-blinded randomised controlled trial in Indonesia for treatment of severe acute malnutrition using rice bran in ready-to-use therapeutic foods (RUTF) | E Ryan	BMS

SESSION 4: TRANSLATIONAL SCIENCE

12:15–3:45 p.m. | LSC 302

Time	Presenter	Topic	Dept.
12:15	Beeney, Amber	Immune responses to radiation therapy and immunomodulation in an oral squamous cell carcinoma mouse model | K Boss	CS
12:30	Dempsey, Jebrail	Investigating *Plasmodium falciparum* isolates in chronic, asymptomatic infections in Homa Bay, Kenya | E Hemming-Schroeder	MIP
12:45	Oldham, Kassandra	Neuroinflammatory and accumulation of misfolded proteins as biomarkers in canine cognitive decline | J Moreno	ERHS
1:00	Smoot, Jacob	Modeling the Effects of an Emerging Toxicant, Wildfire Smoke, on Reproductive Toxicity Using Caenorhabditis elegans | L Montrose	ERHS
1:15	Threatt, Alissa	Omega-3 fatty acid metabolite Resolvin D1 assists recovery after agricultural dust exposure | T Nordgren	ERHS
1:30	Eastman, Ryan	Implantation and early placentation in the mare: the role of kisspeptins in trophoblast invasion | C Magee	BMS
1:45	Kloser, Heidi	Do equine stromal cells maintain pre-injury mobility in a mouse osteoarthritis model? | K Santangelo	MIP
2:00	BREAK		
2:15	Klosowski, Marika	Primary lung fibroblasts promote therapy resistance in co-cultured triple negative breast cancer cells through a juxtacrine, JAK-dependent signaling mechanism | D Regan	MIP
2:30	Kreitner, Kimberly	Characterization of air pollutants at equine racetracks in the United States from 2011-2020 | S Magzamen	ERHS
2:45	Manchester, Alison	Single-cell RNA sequencing analysis of the duodenal mucosa in dogs with chronic inflammatory enteropathy | S Dow	CS
3:00	Owens, Eileen	The cell of origin of canine CD4+ peripheral T-cell lymphoma | A Avery	MIP
3:15	Templeton, Hayley	Sex differences in intestinal Parkinson's Disease pathology | S Tobet	BMS
3:30	Williams, Zoe	Gut microbiome dysregulation is associated with sustained inflammation and differential gene expression in equine progressive osteoarthritis | L Pezzanite	CS

POSTER PRESENTATIONS

SESSION 1 | Odd-numbered posters | 12:15-2 p.m.
SESSION 2 | Even-numbered posters | 2:15-4 p.m.

No.	Presenter	Title | Mentor	Dept.
1	Ahrens, Nicole	Novel extractants for lead (II) liquid-liquid separations | R Sudowe	ERHS
2	Akaraphanth, Mike	CXCR2 perturbation promotes Staphylococcus aureus implant-associated infection | C Gries	MIP
3	Alcantar, Rich	Developing methods to sample potential resuspension of radioactive contaminants near the former Rocky Flats Technical Plant | R Sudowe	ERHS
4	Alsulami, Abdullatif	Cellular Stress Responses Following Wildfire Smoke Exposure in Caenorhabditis elegans | J Moreno	ERHS
5	Awad, Mai	Mechanisms of Piperonyl butoxide cytotoxicity and its enhancement with Imidacloprid and metals in Chinese hamster ovary cells | T Kato	ERHS
6	Ayala, Kevin	A weakening of the blood-brain barrier is identified in a naturally occurring brain aging guinea pig | J Moreno	ERHS
7	Badakul, Gamze	Clustered DNA Double Strand Breaks for Cancer Treatment via Multi-gRNA CRISPR/Cas9 System | J Nickoloff	ERHS
8	Bashor, Laura	Evolution in action: SARS-CoV-2 within-host population expansion, diversification & evolution in tigers, lions and hyenas at the Denver Zoo | S VandeWoude	MIP
9	Behzadi, Elisa	Mercury exposure reveals prey selection in wolves from Denali National Park and Preserve | B Barst, University of Alaska, Fairbanks	Other
10	Berry, Elizabeth	Detection of gastrointestinal parasites in fecal samples from stray cats in Wyoming shelter | M Lappin	CS
11	Bisazza, Katie	Comprehensive Characterization of the Ovariectomized Sheep Model of Osteoporosis: A Systemic, Histomorphometric, and Proteomic Study | J Easley	CS
12	Blair, Noah	Monte Carlo Determination of Detection Efficiency for Portal Monitoring | A Brandl	ERHS
13	Boland, Sean	A liquid suspension of simulated wildfire smoke particulate increases cytotoxicity in RAW264.7 cells | J Moreno	ERHS
14	Bonilla, Andres	Sheep to Human: Unraveling the Translational Potential of Modic Changes in Disc Degeneration | J Easley	CS

No.	Presenter	Title | Mentor	Dept.
15	Bork, Sydney	Detection of a stat5b (n642h) mutation in feline t cell neoplasia with droplet-digital pcr analysis (ddpcr) | A Avery	MIP
16	Bouchet, Courtney	Intrinsic and synaptic properties of posterior hypothalamus neurons in male and female rats | B Myers	BMS
17	Brady, Rachel	CD206 agonism stimulates growth of canine histiocytic sarcoma cell lines | D Thamm	CS
18	Brandon, Jeffrey	Determination of backyard chicken plasma protein reference data using protein electrophoresis | R Moore	MIP
19	Bratlien, Krista	Immune modulating effects of feeding a synbiotic supplement to cats | M Lappin	CS
20	Brehm, Ali	Collective storage of insect specimens increased false positive identification of viral infection | M Stenglein	MIP
21	Brill, Samuel	Development of canine CAR-T cells targeting the disialoganglioside GD2 | D Thamm	CS
22	Brown, Hailey	Validation of a novel preclinical murine model of ankle overuse via mechanical induction | K Sikes	CS
23	Burton, Dylan	Range of motion between adjacent cervical vertebrae in horses with cervical vertebral compressive myelopathy | Y Nout-Lomas	CS
24	Burtz, Madelyn	Radiation Therapy as a Treatment for Urinary Obstruction Secondary to Urothelial Carcinoma in Dogs | T Martin	CS
25	Campbell, Jessi	Testing for Chronic Wasting Disease in Elk on the Western Slope of RMNP by using PMCA and RT-QUIC | M Zabel	MIP
26	Campos, Celine	Gut-Brain-Axis influences Manganese-Induced Neurobehavioral Abnormalities and inflammation in the enteric nervous system | J Moreno	ERHS
27	Carroll, Jordan	Innate fear responses to distal and proximal predator threats | C Vaaga	BMS
28	Castro Romero, Ana Valeria	Effects of acute stress on periaqueductal gray spontaneous synaptic currents | C Vaaga	BMS
29	Chornarm, Nida	Molecular prevalence of select vector-borne pathogens in Thai client-owned anemic dogs in Bangkok and Nakhon Si Thammarat | M Lappin	CS
30	Chunko, Raissa	Effects of trace metals found in asphalt on plutonium uptake on extraction chromatography resins | R Sudowe	ERHS
31	Coats, Cassidy	Implementation of the Ottawa Morbidity and Mortality Model in a Veterinary Teaching Hospital | K Hall	CS

No.	Presenter	Title | Mentor	Dept.
32	Cook, Sara	Determining therapeutic targets for peripheral t cell lymphoma, not otherwise specified in canines | A Avery	MIP
33	Corsato Alvarenga, Isabella	Tolerability of long-term cannabidiol supplementation to healthy adult dogs | S McGrath	CS
34	Coupannec, Maelle	Purification of Ra-226 legacy waste for production of Ac-225: from nuclear waste to cancer treatment | R Sudowe	ERHS
35	Curry, Shay	Evaluating jak/stat inhibitors as therapeutics for feline small cell intestinal epitheliotropic t-cell lymphoma | A Avery	MIP
36	Davis, Kate	Comparability of echocardiographic estimates of stroke volume in healthy dogs | L Visser	CS
37	Day, Amanda	Comparison of a point-of-care assay and a laboratory analyzer for cardiac troponin (cTnI) in guinea pigs (*Cavia porcellus*) | M Sadar	CS
38	Diaz, Anilu	Neutron flux in a howitzer drum and construction of a water-moderated neutron irradiator | R Sudowe	ERHS
39	Dubreuil, Solange	Farmer's perceptions and practices of antimicrobial use and resistance among front range Colorado dairy farm | S Rao	CS
40	Dunn, Brandi	Generating physiologically relevant extracellular vesicles from bovine oviductal organoids | D Tesfaye	BMS
41	Ehrlich, Alexis	Organotypic lung co-culture paradigm to increase T-cell populations *ex vivo* | S Tobet	BMS
42	Glass, Arielle	Testing of Candidate Formulations of CSU's SARS-CoV-2 Vaccine (SolaVAX-CoV-2) for Phase I Human Clinical Trial Use | R Goodrich	MIP
43	Godwin, Lindsey	Causes of mortality in sugar gliders (*Petaurus breviceps*) presented to James L. Voss Veterinary Teaching Hospital, Colorado State University | M Sadar	CS
44	Granger, Kyle	Extended ICU stays, small-gauge catheters, and multiple placements increase risk of peripheral IV catheter complications in cats hospitalized in the critical care unit | L Guieu	CS
45	Haines, Laurel	Characterization of exosome labeling techniques and implications for downstream analysis | D Regan	MIP
46	Hayburn, Regina	A descriptive study of head and neck tumors in young dogs | S Lana	CS
47	Hilliard, Julia	Omega-3 fatty acid docosahexaenoic acid inhibits Staphylococcus aureus growth mediated by FadA β-oxidation | C Gries	MIP

No.	Presenter	Title | Mentor	Dept.
48	Hilty, Samantha	Bluetongue virus surveillance across domestic ruminants in Northern Colorado in 2022 | C Mayo	MIP
49	Hodges, Natasha	Jamaican fruit bats and sarbecovirus susceptibility | T Schountz	MIP
50	Isdale, Rae	Efficacy of a combination of selamectin + sarolaner placed on cats for the prevention of transmission of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* from infected Ixodes scapularis | M Lappin	CS
51	Jakes, Grace	Effects of an innate immune stimulant on dairy calf respiratory health, Salmonella shedding, and cytokine gene expression | S Raabis	CS
52	Jones, Kaylee	Deciphering the interplay of IL-22 and IL-22BP in placental development: implications for maternal-fetal immunomodulation | T Nordgren	ERHS
53	Keke, Chukwudi	Characterizing the nicotine metabolite ratio and its association with sociodemographic and smoking characteristics in HIV-infected smokers in South Africa | J Elf	ERHS
54	Kessinger, Peter	A Habitat Suitability Analysis for Three *Culicoides* Species Implicated in Bluetongue Virus Transmission in the Southeastern United States | S Magzamen	ERHS
55	Khedmatgozar, Chase	Axonal Transport of Alpha-Synuclein Prions | A Woerman	MIP
56	Lee, Rahmi	Co-activation of selective nicotinic acetylcholine receptors improves hippocampal brain rhythms and memory in the mouse of Alzheimer's disease | S Kim	BMS
57	Lessard, Avery	Inhibition of two inflammatory pathways protects from pathological changes due to neurotoxin, MPTP, induced Parkinson's disease | J Moreno	ERHS
58	Longworth, Alyssa	Characterizing tuberculosis immunopathogenesis in guinea pigs: validation of antibodies for cytokine profiling | B Podell	MIP
59	Lowry, Brandon	Using real-time cognitive load measurement to assess instructional design effectiveness | T Clapp	BMS
60	Lukinic, Ema	Infralimbic prefrontal cortical projections to the autonomic brainstem: quantification of inputs to cholinergic and adrenergic/noradrenergic nuclei | B Myers	BMS
61	Maldonado, Pablo	Inactivated *Mycobacterium tuberculosis* as a vaccine strategy for Tuberculosis disease | M Henao-Tamayo	MIP
62	Martinez, Yessica	"The most important thing is to know what to wear when working in the sun": Crop workers' perspectives on workwear | J Rosecrance	ERHS

No.	Presenter	Title | Mentor	Dept.
63	Mazzotta, Megan	Building a one health differential diagnosis: a comprehensive framework for healthcare providers | M Rowh, UC Anschutz	Other
64	McCabe, Kathryn	Elucidating the microcircuitry of the periaqueductal gray | C Vaaga	BMS
65	McGann, Raven	Cerebellar Vermis Stimulation in Real-Time and Conditioned Place Preference Contexts | C Vaaga	BMS
66	Michaelis, Arielle	Investigating the non-conducting role of KV2 at ER-PM junctions, a combined *in vitro* and *in vivo* approach | F Hoerndli	BMS
67	Michalko, Bridget	Evaluation of aging and skeletal maturity of New Zealand white rabbit: a retrospective radiographic analysis | K Sikes	CS
68	Mielnik, Anne	Spatiotemporal Distribution of Metals in Fine Particulates across the Denver Metro Area | S Magzamen	ERHS
69	Mueller, Rebecca	Characterization of a cesium-137 gamma irradiator | R Sudowe	ERHS
70	Muller, Ashley	Helping veterinary clients prepare for disasters for their pets: a CSU pilot study | C Duncan	MIP
71	Nehring, Mary	A Study of Feline Immunodeficiency Virus Prevalence and Expert Opinions on Standards of Care | S VandeWoude	MIP
72	Offermann, Aaron	Canine and human osteosarcoma cell co-culture with primary lung fibroblasts modulates sensitivity to standard-of-care chemotherapy | D Regan	MIP
73	Ogg, Hunter	Transcriptome Reference Map of Adult *Culex tarsalis* Ovaries | R Kading	MIP
74	Oyewole, Emmanuel	Unraveling Toll-like Receptor Dynamics in Attenuating Lung Inflammation from Organic Dust Exposure | T Nordgren	ERHS
75	Patel, Ashana	Transcriptomic responses in synovial fluid cells and circulating leukocytes in horses with progressive osteoarthritis | L Pezzanite	CS
76	Plaisance, Cody	Transcriptomic response of equine synovial tissues following immune conditioned cellular therapy to treat septic arthritis | L Pezzanite	CS
77	Potter, Ashley	Characterizing the FAT-1 mouse strain in a non-surgical model of ACL rupture | T Nordgren	ERHS
78	Quintana, Joedy	Preparing Pets and their People | C Duncan	MIP
79	Ramirez, Gabriela	*Anopeheles stephensi* mosquitoes: interrogating their metabolic profile and cryopreservation methods | K Dobos	MIP

No.	Presenter	Title | Mentor	Dept.
80	Risen, Sydney	Translational inhibition of NLRP3 and NF-κB is protective in Experimental Autoimmune Encephalomyelitis (EAE) Mouse Model of Multiple Sclerosis | J Moreno	ERHS
81	Roach, Regan	The autism-associated loss of δ-catenin functions disrupts social behavior | S Kim	BMS
82	Rockow, Meagan	Current Antimicrobial Use in Horses Undergoing Exploratory Celiotomy: A Survey of Board-Certified Equine Specialists | L Pezzanite	CS
83	Rogers, Heather	Transcriptomic Insights into Advanced Maternal Aged Granulosa Cells Emphasizes Inflammation and Immune Response | D Tesfaye	BMS
84	Roh, Scott	Prenatal exposure to valproic acid reduces synaptic δ-catenin levels and disrupts ultrasonic vocalization in neonates | S Kim	BMS
85	Sanchez, Valeria	Air quality at United States' equine eventing competitions | C Duncan	MIP
86	Schmelzer, Camryn	Investigating the origin and nature of half-crossover cascades in *Saccharomyces cerevisiae* | L Argueso	ERHS
87	Shelton, Kimberly	Uncovering biomarkers to improve identification of latent tuberculosis infection and prediction of disease outcome | K Dobos	MIP
88	Shirley, Payton	Validation of Cannabigerovarinic acid (CBGVA) Pharmacokinetics in C57BL6 mice | J Moreno	ERHS
89	Siegenthaler, Britta	Development and validation of a new coma scale and long-term outcome scoring system for dogs with Traumatic Brain Injury (TBI) | K Hall	CS
90	Singh, Benjamin	Pilot: An approach to assessing enrichment items as modifiers of daily activity in laboratory guinea pigs | M Sadar	CS
91	Stephenson, William	Determination of strontium-90 and cesium-137 in freshwater fish near the Fukushima Daiichi Nuclear Power Plant | R Sudowe	ERHS
92	Stratton, Claire	A novel co-culture model highlights extrinsic modulation of TNBC therapy responses by primary lung fibroblasts | D Regan	MIP
93	Sullivan, Caroline	Sustainability at the CSU veterinary teaching hospital: identifying areas of improvement to inform future action items | C Duncan	MIP

No.	Presenter	Title | Mentor	Dept.
94	Svenson, Gwen	The benefits of mentoring underserved high schoolers in veterinary medicine and One Health through a free, experiential college course | C Magee	BMS
95	Kofron, Kristine	Parvovirus antibody titers in sera from dogs in a community based blood donor program | M Lappin	CS
96	Tees, Lily	Optimizing an enzyme-linked immunosorbent assay for transgelin-2 to diagnose feline infectious peritonitis | G Dean	MIP
97	Terry, James	Cellular decapping enzyme Dcp2 implicated in the production of subgenomic flavivirus RNA | B Geiss	MIP
98	Thompson, Riley	Equine endometrial organoids as an *in vitro* model for endometritis to trial novel therapeutics | F Hollinshead	CS
99	Tyer, Leo	Characterizing abnormal cases of chronic wasting disease observed in Norwegian moose possessing a lysine to glutamine polymorphism in the prion protein at codon 109 | G Telling	MIP
100	Van Zeeland, Emily	Investigating the Influence of Intra-articular Estrogen on Post-Traumatic Osteoarthritis Following Mechanical Anterior Cruciate Ligament Injury in a Mouse Model | K Sikes	CS
101	Viola, Lindsey	Veterinary anesthesia: an opportunity to reduce the environmental footprint of clinical care | C Duncan	MIP
102	Walrond, Katy	Investigating glial fibrillary acidic protein to diagnose and predict outcome in canine traumatic brain injury | T Webb	CS
103	Wittenstein, Jessica	Enhancing the antioxidant capacity of bovine embryos against oxidative stress using quercetin supplementation | D Tesfaye	BMS
104	Zaiger, Megan	Influence of pH and temperature on the adsorption of radiocesium on Prussian Blue coated detonation nanodiamonds | R Sudowe	ERHS
105	Zbysinski, Tony	Ionizing radiation and mortality: unraveling neurocognitive risks in a worker cohort | A Neophytou	ERHS
106	Zisumbo, Raquel	Assessment of wildfire smoke exposure on bovine reproductive health | L Montrose	ERHS
107	Zook, Sophie	Epizootic hemorrhagic disease virus (EHDV) prevalence in white-tailed and mule deer in northeastern Colorado | C Mayo	MIP
108	Ferrari, Giulia	Clinical signs of HPAI in wild birds presented to US rehabilitation facilities | B Wolfe	CS

1. Peripheral Mycobacterium tuberculosis Infection Results in Neuroinflammation and accumulation of Misfolded Proteins in Non-Human Primates

Isla Anderson, Amanda Latham, David F Ackart, Randall J Basaraba, Julie A Moreno

Tuberculosis (TB) is a bacterial infection caused by *Mycobacterium tuberculosis* (Mtb) and affects approximately ten million people each year worldwide. Primarily caused by infection of the lungs, TB induces a robust peripheral immune response of cytokine producing cells. Interestingly, epidemiological studies show associations between TB and increased risk for neurodegenerative diseases, including Parkinson's Disease (PD) and dementia, as well as decreased cognition in patients co-infected with TB and Human Immunodeficiency Virus (HIV). These correlations exist without diagnoses of central nervous system (CNS) Mtb infection or tuberculosis meningitis (TBM), where infection disseminates to the brain. We have previously shown using guinea pigs, which are a pathologically relevant model of human disease, that peripheral Mtb infection results in neurological changes. These include glial reactivity, neurotoxic protein aggregation, and neuronal loss. Guinea pigs are a relevant animal model, because their cellular responses to Mtb infection parallel that of humans, however, non-human primates (NHP) are considered to have greater translational potential, because the NHP shares similar genetics, anatomy, and physiology to that of humans. In our current study, brains from NHPs peripherally infected with two common laboratory strains of Mtb were examined. Our preliminary data shows that animals exposed to Mtb display a neurotoxic brain phenotype compared to uninfected controls, including increased microglia and C3/S100b positive activated astrocytes. Through these data, we demonstrate the translational capacity of our previous findings, supporting our hypothesis that TB disease may contribute to long-term cognitive decline in human patients. Further analysis of these NHP brains will also deepen our understanding of how the peripheral immune response affects the brain. Research funded by the Boettcher Foundation and the Department of Microbiology, Immunology and Pathology at Colorado State University.

Undergraduate Student/ ERHS

2. Haploinsufficiency of the autism-associated δ-catenin mutation is sufficient to induce social dysfunction in mice

Victoria Aragon, Emma Hinchliffe, Seonil Kim

Social behavior is essential to survive for many species, and various mental disorders have social impairment as a primary symptom. Research suggests that synaptic activity and signaling can regulate social behavior. However, the links between synaptic regulation and social behavior are not completely understood. δ-catenin functions as an anchor for the glutamatergic AMPA receptor (AMPARs) to regulate synaptic activity in excitatory synapses. Mutations in the δ-catenin gene are found in autism patients from multiple families and induce a loss of δ-catenin functions at excitatory synapses, which is thought to be the etiology of autism in people.

In fact, patients heterozygous for deletions or loss-of-function variants of the δ-catenin gene exhibit a variety of features of autism. Therefore, δ-catenin heterozygous mutant is a valuable loss-of-function model to investigate ASD pathophysiology caused by δ-catenin haploinsufficiency. Here, we used mice harboring one copy of human autism-associated δ-catenin missense mutations, a glycine 34 to serine (G34S), to address whether δ-catenin haploinsufficiency that mimics the human condition is sufficient to induce social dysfunction in animals. To test this, the reciprocal social interaction test to examine social interaction between two freely moving mice was performed. We found that the total number of contacts and total duration of contacts were significantly reduced in both female and male G34S heterozygous mice. Additionally, the open field test showed normal locomotor activity and anxiety levels in these mice. Therefore, we discover that δ-catenin haploinsufficiency is sufficient to induce social dysfunction in mice. Research funded by NIH and the Boettcher Foundation.

Undergraduate Student/ BMS

3. Evaluating the presence of Chronic Wasting Disease in free-range White-tailed deer in Arkansas

Lauren S. Bennett, Nathaniel D. Denkers, Mark G. Ruder, Michael J. Chamberlain, Candace K. Mathiason

Chronic wasting disease (CWD) is a prion disease affecting free-range and captive cervid populations in North America, Europe, and Asia. CWD is the result of an accumulation of misfolded, pathogenic prion proteins within lymphoid and neural tissues. The deposition of prions in the brain ultimately leads to vacuolation, spongiosis, and death. CWD is a concern in wild cervid populations as the precise route of transmission and progression throughout the body is unknown. Understanding the pathogenesis of CWD, from acquisition to shedding and transmission, will fill gaps in our knowledge about the nature and mechanisms of CWD transmission. Experimentally, white-tailed deer models have been used to determine the progression of CWD prions throughout the body, providing information about early and late-stage disease progression. To understand how CWD affects free-ranging native populations, tissues harvested from white-tailed deer in Arkansas (2021-2024) will be analyzed by enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC) for the presence of CWD deposition. Findings will be used to determine CWD prevalence for the study area, and the stage of disease progression for each deer. Preliminary data from IHC testing on terminal tissues has confirmed that prion deposition can be detected in retropharyngeal lymph node, tonsil, and obex tissues. Analysis from an additional 120 terminal tissues will be compared to the ELISA results obtained from the Southwest Cooperative Wildlife Disease Study (SCWDS) to corroborate findings. The overall goal for this work is to provide a clearer picture of CWD prevalence and disease progression to wildlife agencies that can be used to better inform disease management decisions. Funded by the Arkansas Game and Fishing Commission (AGFC), and the National Institutes of Health (NIH).

Undergraduate Student/ MIP

4. Selective cholinergic activation prevents memory loss and the *in vivo* growth of amyloid plaques in Alzheimer's disease

Ellison Black, Rahmi Lee, Michael Doolittle, Seonil Kim

Alzheimer's disease (AD) is the most common form of dementia with no known cause and cure. Studies suggest that one of the main causes of AD is disruptions in synaptic activity of GABAergic inhibitory interneurons by beta-amyloid peptide (Aβ). This in turn decreases inhibitory activity to increase excitation in pyramidal excitatory neurons in the hippocampus, resulting in network hyperexcitability. Hyperexcitability in the hippocampal network also promotes Aβ secretion and accumulation, leading to the formation of amyloid plaques, a central pathology of AD. This suggests that the Aβ-induced reduction of hippocampal inhibition is a crucial trigger for the development of AD. Therefore, enhancing hippocampal interneuron activity is thought to be neuroprotective against AD. We thus hypothesize that Aβ-induced hippocampal hyperexcitation promotes the in vivo rapid growth of amyloid plaques, which can be reversed by increasing hippocampal inhibition. To activate hippocampal inhibition, we injected drugs to stimulate α7- and α4β2-nicotinic acetylcholine receptors (nAChRs) into 5-month-old amyloid pathology model (5XFAD) mice. Hippocampal sections from these mice were stained with Thioflavin S to visualize amyloid plaques. We found that in vivo co-stimulation of α7- and α4β2-nAChRs significantly reduced the total area and average size of amyloid plaques in the 5XFAD hippocampus when compared to the control hippocampus. This suggests that co-activation of these two receptors significantly reduces the growth of amyloid plaques in 5XFAD mice by preventing hyperexcitation in hippocampal pyramidal cells. Funded by NIA, the Boettcher Foundation and BrightFocus foundation.

Undergraduate Student/ BMS

5. Ketamine reverses chronic stress-induced mental disorders via the expression of Ca2+-permeable AMPA receptors in mice

Evelina Bouckova, Paige E. Vetter, Joshua C. Flowers, McKennon J. Wiles, Madison H. Wustrau, Rahmi Lee, Seonil Kim

Both preclinical and clinical studies demonstrate that chronic stress reduces AMPA Receptor (AMPAR) subunit GluA1 levels in hippocampal synapses, while there are conflicting results describing alterations in hippocampal GluA2 levels under chronic stress. These results suggest that the stress-induced decrease in hippocampal GluA1 levels is correlated with both weakened excitatory synaptic transmission and altered hippocampus-dependent behaviors in chronic stress. Importantly, we have revealed that low-dose ketamine rapidly induces the expression of GluA1-containing, GluA2-lacking Ca2+-Permeable AMPARs (CP-AMPARs), a subtype of AMPARs that have larger single channel conductance, in the hippocampus. We have further shown that this ketamine-induced CP-AMPAR expression enhances glutamatergic synaptic strength in hippocampal neurons, which allows animals to exhibit less anxiety- and depression-like behaviors. Our new findings further demonstrate that low-dose ketamine treatment can reverse disruptions in hippocampus-dependent fear memory and social behavior caused by chronic restraint stress (CRS) in mice. Research also shows that ketamine-induced restoration of impairments of AMPAR-mediated synaptic transmission and behaviors in stressed animals is associated with an increase in synaptic GluA1 expression in the hippocampus. Notably, the hippocampus is one of the key brain regions controlling social behavior and learning and memory. Moreover, an increase in hippocampal activity reverses stress-induced memory impairment, social dysfunction, and mood disorder-linked behaviors. More importantly, a recent study shows that the hippocampus is selectively targeted by low-dose ketamine. These existing data and our findings show that ketamine at the low dose rapidly induces the expression of CP-AMPARs in the hippocampus, which in turn enhances synaptic strength to reverse hippocampus-dependent behavioral dysfunctions in chronically stressed animals.

Undergraduate Student/ BMS

6. An exploration of the stability and integrity of ready-to-use therapeutic foods in Indonesia

Emma S. Bovaird, Annika Weber, Elizabeth Ryan

Rice bran is a fibrous, nutrient-rich by-product of the milling of brown rice to produce white rice. Given the high global consumption and production rates of white rice, rice bran is readily available and has the potential to be utilized as a relatively affordable ingredient. Recently, rice bran was added for the first time to ready-to-use therapeutic foods (RUTFs), which are energy dense foods used to combat cases of severe acute malnutrition (SAM). RUTF with 5% rice bran and the same RUTF without rice bran were recently implemented in a double-blinded, randomized controlled clinical trial on gut microbiota-targeted treatment of SAM in Jember, Indonesia. Here, we aim to determine the stability and integrity of the nutrient composition of the two RUTFs over time. This study also considers the nutritional impact of the addition of rice bran to RUTFs and its potential to lower the amount of vitamin and mineral premix needed for each product. To determine the effects of time and heat exposure on the nutrient stability of the products, the macro- and micronutrient composition of the RUTFs from the clinical trial with and without rice bran will be analyzed at baseline and compared to RUTFs that have spent a period of 3.5 months in the field. Additionally, these RUTFs from the field will be compared to RUTF test products that contain no premix and rice bran, and no premix and no rice bran to act as the control. The analysis will include the consideration of macronutrients, dietary fiber, and B vitamins, among others. The goal of this study is to consider the viability of utilizing rice bran in RUTFs in Indonesia and the potential to decrease product cost while maintaining its nutritional value. Funded by the Thrasher Research Fund.

Undergraduate Student/ ERHS

7. Exploring CYP6AG7's role in pesticide resistance in *Aedes aegypti*

Isabelle Byfield, Karla Saavedra Rodriguez

Aedes aegypti is the major mosquito vector of dengue, chikungunya, and Zika virus. Because of lack of vaccines against these arboviruses, prevention and control has relied in suppressing mosquito population during disease outbreaks. Use of pyrethroid insecticides has been the main method of mosquito suppression since 1999. Unfortunately, prolonged and heavy use of pyrethroids has resulted in widespread pyrethroid resistance in mosquito populations. One of the mechanisms reported in resistant mosquitoes is enhanced insecticide metabolism by the mosquito's detoxification genes, including cytochrome P450's (CYPs), carboxyl-esterases and glutathione transferases. These proteins detoxify the pesticide into nontoxic metabolites. Exome-wide association mapping identified single nucleotide polymorphisms (SNPs) in a cluster of CYP6 genes (CYP6AG-3, -4, 7 and –8) to be highly associated with pyrethroid resistance. In this study, we performed Sanger sequencing to identify potential SNPs in CYP6AG7 and then designed allele-specific melting curve primers to genotype mosquitoes with those mutations. Our main goal is to test these SNPs as biomarkers of cytochrome-mediated resistance in mosquito populations.

Undergraduate Student/ MIP

8. Factors influencing the pathogenesis of Tuberculosis meningitis in mice

Daryl Conner, Heidi Kloser, Sasipha Hokeness, Faye Lanni, Taru Shikha S. Dutt, Elizabeth Julie Creissen, Elizabeth Marie Dorst, Kristina Nguyen Tran, Brennen Tyler Troyer, Jessica Ann Glycenger, Andres Obregon Henao and Marcela I Henao Tamayo

Tuberculosis Meningitis (TBM) is a severe extrapulmonary manifestation caused by *Mycobacterium tuberculosis* (Mtb), particularly affecting children under 5 with ~ 50% mortality rate. Limited and costly models exist for most TBM studies so this study was aimed to create an affordable and user-friendly model for comprehensive research on this devastating disease. TBM is characterized by inflammation of the meningeal membranes surrounding the brain and spinal cord. We worked with C57/BL-6 mice as our animal model and utilized the blood-brain barrier (BBB) disruptive properties of drugs, Cyclopamine and GDC-0449. Mice were infected via low-dose aerosol with Mtb strain HN878. The animals were then treated with the BBB-disrupting drugs post-infection. Animals were monitored weekly for behavioral/motor changes with ANY-maze, a cage monitoring system. Brain, lungs, and spleen were collected at necropsy for disease burden and pathological analysis. We observed behavior/motor changes in some of the groups as the study progressed. There were also measurable colony-forming units (CFUs) in the spleen, lungs, and brains of the mice. The results from our BBB-disrupting drugs groups indicated successful dissemination of Mtb into the brain. Aside from a few of the measures, we did not see the behavioral changes that we were expecting to see in the animals based on previous work with rabbits. The histopathology of the brain is under analysis. Evaluating the tissue inflammation and immune responses, along with increasing the longevity of the study, may also prove useful in understanding TBM in mouse models. Funded by 2022 Monfort professor award.

Undergraduate Student/ MIP

9. Unraveling the IL-24 Molecular Mechanisms of Dust-Induced Lung Inflammation in Mice

Ashley DeBie, Melea Barahona, Tara Norgren

Inhaled toxicants are recognized as a major contributor to lung inflammation and tissue damage. Repair mechanisms within the lung epithelial barrier aid in maintaining tissue homeostasis and protect against injury. The epithelial-to-mesenchymal transition (EMT) allows cells to gain proliferative abilities, and influences cancer formation and metastasis. Inhalation of dust further exacerbates this process. Additionally, in cellular immunity, macrophages release cytokines to regulate inflammation and recruit other immune cells in response to foreign insults. Interleukin-24 (IL-24) is a multifunctional IL-10 family cytokine, known for its various roles in inflammation. Elevation of this cytokine has been found in autoimmune diseases, such as pulmonary fibrosis[1]. Interestingly, this cytokine also mediates an inflammatory response in wound healing. Despite its similarity to IL-22, IL-24 has few studies on its role in the signaling process following dust exposure (DE). The goal of this research is to provide evidence of the role of IL-24 in this process. To investigate these gaps in knowledge, wildtype (WT) C57BL/6 mice were instilled with an aqueous dust extract (DE) and sacrificed at time points of 24 hours, 48 hours, 5 days, and 3 weeks. Bronchoalveolar lavage fluid (BALF) and lung tissue samples were collected and analyzed for the cytokines IL-22 and IL-24 via sandwich ELISA methodology, found to both be upregulated in response to dust exposure. IL-24 appears to have a larger role in the inflammatory response with increased IL-24 concentrations in the 5 day DE female mice, showing differential secretion between sexes in response time. Further investigation into IL-24 activity after inhaled DE and sex differences in the production of this cytokine is ongoing. Research is funded by NHLBI (R01HL158926 to TMN) and T32GM132057 (qCMB Training Grant) and is overseen by Dr. Tara Nordgren.

Undergraduate Student/ ERHS

10. Antiviral roles of sphingolipid metabolism

Kaitlyn Dirks, Hannah Laurence, Laura St. Clair, Paul Soma, Rushika Perera

Flaviviruses have previously been shown to alter host cell lipid metabolism to support the extensive membrane morphological changes they induce for virus replication complex assembly. Ceramide, a molecule that belongs to a class of bioactive signaling molecules called sphingolipids, is elevated in both human and mosquito hosts during infection with dengue virus, serotype 2 (DENV2). This sphingolipid forms both a structural component of host cell membranes and is also utilized in cellular signaling pathways. Ceramide is produced by three pathways: the sphingomyelin pathway, the *de novo* biosynthesis pathway, and the salvage pathway. Alterations in in these pathways have been associated with pathology resulting from flaviviral infections. Preliminary loss of function studies of enzymes in the sphingolipid pathway in human hepatoma (Huh7) cells have identified that 3-Ketodihydrosphingosine Reductase (KDSR) and UDP Glycosyltransferase 8 (UGT8), involved in the synthesis of glycosylated ceramides are antiviral. In addition, each enzyme has been shown to bind to flaviviral non-structural protein 4A (NS4A) in affinity purification-mass spectrometry analysis. We hypothesize that flaviviruses alter expression of enzymes involved in sphingolipid metabolism to confer an advantage over the host. We validated the loss of function studies using siRNA knockdown in human hepatoma (Huh7) cells followed by infection with dengue virus, serotype 2, or Zika virus, and assessed the effects on viral replication. We also overexpressed each enzyme in Huh7 followed by infection with DENV2 and showed a decrease in viral replication, confirming the antiviral phenotype. Confocal microscopy was used to assess co-localization of KDSR and UGT8 with flaviviral nonstructural proteins. We are currently investigating the antiviral mechanisms of these host proteins. These studies will help deepen our understanding of the mechanisms flaviviruses employ to gain an advantage over the host cell during infection. Funded by the Anschutz Family Foundation and the CVMBS College Research Council.

Undergraduate Student/ MIP

11. Neuropathological Changes in the Aged Canine with Canine Cognitive Dysfunction

Tatum Flatt, Masa Ukai, Kassandra Oldham, Julie Moreno, Stephanie McGrath

Canine cognitive dysfunction (CCD) syndrome is a prevalent neurodegenerative disease that causes cognitive decline primarily in dogs aged 8 years and older with approximately 35% of canines affected. Due to the similarities between the pathogenesis of CCD and the human equivalents of neurodegenerative diseases such as Alzheimer's disease (AD), canines serve as ideal animal models. To better understand the natural disease development of CCD, canine brains are dissected post-mortem and stained using the immunohistochemical (IHC) staining process to image and cell count biomarkers of interest. Brain regions most affected by CCD and AD are the frontal cortex and hippocampus. The frontal cortex plays an important role in cognition and higher-level thinking, containing the frontal lobe that is responsible for memory, attention, and planning. Hippocampus functions include creating the memory circuit, allowing for learning and memory encoding. These two regions are of particular interest in regard to CCD due to their role in cognition. The dissections allow for direct comparison between healthy aged brains and brains with diagnosed CCD by providing quantification with cell counts of CCD biomarker accumulation, including misfolded proteins, gliosis, and neuroinflammation. Results suggest an increase in hyperphosphorylated tau protein at multiple sites, including threonine-217 and threonine-231, amyloid beta plaques, and neuroinflammation in the aged CCD positive canine when compared to the aged negative CCD canines, but no significance calculations can be performed due to the small sample size. As the study population increases with more dogs enrolled, statistical significance can be drawn from the data to establish biomarkers as causative agents of CCD. Additionally, more antibodies can be used to assess if other markers of cognitive decline and related disease similarly seen in humans, such as alpha synuclein, as well as adding environmental considerations for exposures and living conditions.

Undergraduate Student/ ERHS

12. Investigating SARS-CoV-2 evolution and transmission in lions, tigers, and hyenas at the Denver zoo using low-copy late-stage nasal swabs

Michelle A. Galvan, Laura Bashor, Mark Stenglein, Emily Gallichotte, Katelyn Erbeck, Lara Croft, Katelyn Stache, Jimmy Johnson, Kristy Pabilonia, Sue VandeWoude

Many non-human animals are susceptible to SARS-CoV-2, in particular members of the family Felidae. For non-domestic felids, this is most evident in places such as zoos where interactions at the human-wildlife interface are more frequent. In these environments, viral evolution and cross-species transmission of SARS-CoV-2 have not been deeply investigated. In the fall of 2021, an outbreak of SARS-CoV-2 occurred in animals housed at the Denver Zoo in Colorado, USA. Eleven lions, two tigers, and four hyenas were observed with a range of symptoms including coughing, sneezing, lethargy, and nasal discharge production. Repeated nasal swabs were obtained from all the animals and evaluated by real-time PCR (RT-PCR). Every individual tested positive for SARS-CoV-2 at least once, except for two hyenas. Out of 114 total samples collected, 50 were classified as "inconclusive" (not definitively positive or negative) following RT-PCR. The majority of the latter were collected toward the end stages of infection after multiple positive tests. To further investigate virus within-host evolution and map out an infection timeline we screened the "inconclusive" samples for potential virus genome sequencing. Of 50 samples, 10 (20%) resulted in positive amplification by tiled amplicon PCR. Seven samples contained sufficient DNA for library preparation in which PCR amplicons received adaptors and unique dual indexes prior to Illumina next-generation sequencing. Our findings reveal the presence of SARS-CoV-2 genomic material in low-copy late-stage nasal swabs from infected animals, including the first reported cases of SARS-CoV-2 infecting the Hyaenidae family. Analysis of the next-generation sequencing data will provide further insight into how this virus evolves during novel cross-species transmission. Research was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number T32AI162691 and the National Institute of General Medical Sciences of the National Institutes of Health under Grant Number T34GM140958.

Undergraduate Student/ MIP

13. Identifying Plasma Biomarkers for ALS Utilizing Human and SOD1^{G93A} Mouse Model

Nizhoni Hatch, Angel Grace Leslie, Ran Wei, David Bradford, Kathleen Rodgers, Philida Charley

Amyotrophic lateral sclerosis (ALS) is a rare, progressive neurodegenerative disease that leads to the death of motor neurons resulting in paralysis and death. Currently, there is no cure or effective therapy to reverse disease progression. The diagnosis of ALS relies on clinical examination and ruling out other diseases through a variety of muscle and imaging tests. The identification of biomarkers could be used to develop tests to confirm ALS diagnosis and estimate disease progression. This study aimed to identify blood-based biomarkers and their representative biological pathways to characterize ALS by analyzing the significant plasma protein changes in the SOD1^{G93A} mouse model compared to plasma from human ALS patients. A comprehensive proteomic analysis was conducted on the SOD1G93A mice using the SomaLogic platform at different timepoints; 1) presymptomatic stage, 2) symptom onset, and 3) disease endstage in both healthy wild-type controls and SOD1^{G93A} mutant mice. The animal protein profiles were associated with human ALS samples in a parallel study to identify translatable biomarkers. Thirty-one significant proteins were associated with disease progression in mice and 401 in the human data. There were seven proteins shared between the mouse and human data. The related biological pathways were identified using the online data platform, Reactome, with the most significant relevant pathways involving extracellular matrix organization and laminin interactions. In the future, these proteins will be a target of interest for drug development to assess target engagement in slowing ALS progression. This research addresses the critical need to develop reliable, blood based biomarkers that not only confirm an ALS diagnosis but also estimate disease progression. This research was funded by Diné College & the University of Arizona's fellowship program, Undergraduate Readying for Burgeoning Research for American IndianNeuroscientists (URBRAIN) (grant number: R25NS107185), and Colorado State University's McNair Scholars Program.

Undergraduate Student/ MIP

14. Investigation of health education resources for students at Colorado State University regarding regional vector-borne disease

Emma Hebert, Sheryl Magzamen

Every year, about 30,000 individuals flock to Fort Collins, CO to attend Colorado State University, approximately 25% of these individuals are from another state and 2,500 are from a country outside of the U.S. Every state, region, and country are unique for a number of reasons, including for their prevalence of specific diseases. For some 30% of out-of-state students who travel to Fort Collins every year, their knowledge of the unique diseases in Colorado or the Mountain West region may be limited. Additionally, individuals who have lived in Colorado for an extended period of time and are deemed in-state students may also have limited knowledge about these diseases due to lack of proper news coverage, education, or access to information. To determine if CSU has been providing information to their students regarding regional disease in Northern Colorado, in-depth searches of their main website, health network website, YOU@CSU, and environmental health website occurred and conversations with representatives from YOU@CSU, health network, and president's office were had. Additionally, searches of websites for the Larimer County Public Health and Environment (LCPHE) and Colorado Department of Public Health and Environment (CDPHE) occurred. There was no information found on any CSU-related website regarding region-specific diseases such as Hantavirus, West Nile, Plague, or tick-borne diseases such as Colorado Tick Fever, Tick-borne Relapsing Fever, or Tularemia. On the LCPHE and CDPHE websites, there was mention of region-specific diseases, but they were not comprehensive, attractive, or tailored to University students or new Colorado residents. CSU does not have information regarding the region-specific diseases in Colorado and the information provided by the LCPHE and CDPHE are limited. Therefore, students may be unaware of the diseases they could be exposed to and are unable to make educated risk-assessments or decisions about their health.

Undergraduate Student/ ERHS

15. Characterization of the locomotor changes and neuroinflammatory phenotypes in Gerstmann-Straussler Scheinker (GSS), a familial prion disease

Caleb Hopkins, Sean Boland, Hyatt Vincent, Glenn Telling, and Julie Moreno

Gerstmann-Straussler Scheinker (GSS) is an inheritable prion disease. Although it is rare, it is 100% fatal, if diagnosed. Familial prion disease accounts for approximately 10-20% of prion disease cases and is caused by autosomal dominant mutations found in the human prion gene (PRNP). Gerstmann-Straussler Scheinker (GSS) syndrome is most commonly due to a PRNP mutation at codon 102 with an amino acid substitution of leucine to proline. This creates the misfolding of the native prion protein structure PrP^{C} into the pathological conformation of PrP^{Sc}, which is followed by accumulation and aggregation within the brain. The accumulation of PrP^{Sc} is believed to drive various cellular stress mechanisms that inevitably results in cortical and cerebellar atrophy, which leads to signs and symptoms, including motor abnormalities and dementia. However, like all prion diseases, the exact extent of cellular mechanisms behind GSS disease pathology is not fully understood. This creates a necessity to investigate GSS pathogenesis. We utilize transgenic GSS (TgGSS) mice to follow the progression of the disease from 12 weeks until the mice were terminally ill and succumbed to the disease, about 23 weeks of age. We assess neuroinflammation by immunohistochemistry (IHC), as well as motor deficits by rotarod. We hypothesize with motor changes we will identify neuroinflammation and cerebellar neuronal loss. Our preliminary results show a steady trend of time spent on the rotarod from 12 to 17 weeks of age. This was followed by a significant decrease at 17 weeks and followed by a steady decline until mice were terminal. This highlights motor loss during disease progression. We will continue to analyze IHC to assess neuroinflammation with S100b for astrocytic activation and IBA1 for microglial number. Understanding the neuroinflammatory phenotypes of this familial prion disease will allow us to determine if avenues of possible mitigation of the fatal neurodegenerative disease.

Undergraduate Student/ BMS

16. NPY intracranial ventricular injection suppresses LH pulsatile secretion in adult female mice

Kyla D. Jacobs, Lauren Young, Evan Hurtado, Richard McCosh

Stress inhibits reproductive function through suppression of luteinizing hormone (LH) secretion. LH pulses support steroidogenesis and gametogenesis in mice and humans. Many signaling molecules have been identified in hypothalamic nuclei involved in LH pulse regulation, one of which is neuropeptide Y (NPY), which has been shown to centrally suppress feeding behavior and increase glucocorticoids. The objective of this study was to determine if central NPY is sufficient to suppress LH pulsatile patterns in adult female mice. These wild-type mice were tail-handled for 5 weeks and ovariectomized 10 days before the experiment. Blood samples were taken every six minutes for 1.5 hours, followed by freehand intracranial ventricular (ICV) injection of NPY (500 pmol) or saline (3 μL) into the lateral ventricle while under anesthesia. After 30 minutes, blood samples were taken once again every six minutes for 1.5 hours. An ultra-sensitive ELISA was utilized to determine the concentration of LH in each blood sample. It was found that all animals exhibited LH pulses pre-injection and all saline-treated animals had LH pulses post-injection. NPY-treated animals displayed a split response: two had a dramatic decrease in LH pulses (mean of 5.0 ± 1.4 pulses pre-injection, 0.5 ± 0.5 post-injection) and two had pulses post-injection (likely due to injection not into the ventricle, so their data have been omitted from analysis). Saline-treated mice had a mean LH concentration of 6.3 ± 0.4 ng/mL pre- and 5.0 ± 0.3 ng/mL post-injection, while NPY-treated mice had 7.6 ± 0.4 ng/mL pre- and 2.2 ± 0.01 ng/mL post-injection. These results support our hypothesis that NPY is important for LH pulse suppression in mice, which presents new avenues of research into fertility disorders. Funded by NIH R00 HD104994.

Undergraduate Student/ BMS

17. Dietary inclusion of rice bran increases functional food properties of meals and snacks

Maya Jones, Lei Zhang, Madison Tipton, Bridget A. Baxter, and Elizabeth P. Ryan

Rice bran is a functional food that has been shown to control and prevent metabolic disturbances and inflammation involved in chronic diseases, such as obesity, diabetes, cardiovascular disease, and colorectal cancer. This study identified the nutritional and metabolomic composition of five meals/snacks (baked pasta marinara, Margherita pizza, blackberry cobbler, caraway crackers, and strawberry-pineapple smoothie) used in a human clinical trial. Each meal/snack contained 15g of heat-stabilized rice bran (control foods had 0g of rice bran for comparison). Nutrient, metabolite, and bioactive profiles were analyzed using an integrated food and nutritional metabolomics approach, to evaluate the nutrient profiles to determine if adding rice bran enhanced the nutritional content and to document the presence or absence of specific metabolites that may be linked to improving gut health. The relative abundance of 22 distinct metabolites was higher in meals/snacks containing rice bran compared to control. Nine of these 22 metabolites have reported evidence to prevent or control the progression of diabetes in the literature. Nutritionally beneficial Linoleic acid and fiber levels were higher in foods with added rice bran. 10 of the 22 metabolites were impacted by cooking methods or the presence of microbes. Pizza Margherita contained yeast and strawberry-pineapple smoothies contained L. acidophilus that diminished food metabolite composition. These findings enhanced our understanding of the functional food properties of rice bran incorporated into meals/snacks and can inform future development of food products for use in clinical trials that may have benefits for chronic disease control and prevention efforts. Funded by the USDA National Institute of Food and Agriculture.

Undergraduate Student/ ERHS

18. Testing the aerosolization of bacteria from an inoculating loop

Madeline Judson, Doreene Hyatt, Claudia Gentry-Weeks

The flaming of an inoculation loop is an essential step in several microbiology laboratories and procedures. However, the exact process is not uniform with some individuals 'flaming' a loop in different orientations. This study aims to test whether the sterilization of an inoculation loop is more effective when the loop enters a flame from the tip or when it enters from the base. It is our belief that given how common this technique is in microbiology procedures, it is vital to understand the difference the direction has of flaming a loop, as well as the potential implications of said direction.We tested this by flaming a loop containing a known bacterial isolate in both orientation over a large agar plate, or 'Mega plate' to check for potential growth from the aerosolization of the bacteria on the loop. These 'Mega plates' are square plates, 23 inches each side, and have a hole in the middle of them for where the bunsen burner will be placed. We additionally tested the effect of flaming an inoculating loop from a liquid culture vs. a bacterial colony, and the Gram stain identity of the bacteria growing that is being sterilized. We believe that the orientation of the inoculation loop when entering a bunsen burner will not have an effect on the aerosolization of bacteria regardless of said bacterias Gram identity. Through the results of this study we aim to contribute to a further understanding of everyday microbiology laboratory techniques, to better the laboratory experience for researchers, students, professors, and others.

Undergraduate Student/ MIP

19. Extracellular Vesicles from Oviductal Organoids Capacitate Bull Sperm Enabling Fertilization *In Vitro*

Marcie Jung, Mindy Meyers, Brandi Dunn, Fiona Hollinshead, James K. Graham

Frozen semen is widely used with good success in the dairy and in beef industry using artificial insemination (AI). However, when used for vitro fertilization (IVF) frozen-thawed (FT) bull semen produces variable results. Cryopreservation damages sperm plasma membranes, increases membrane fluidity, changes sperm binding to the zona pellucida, and induces premature acrosome exocytosis, which reduces longevity and viability. Extracellular vesicles (EVs), in seminal plasma (SP) and oviductal (O) fluids, can affect sperm via their cargo to enhance sperm motility, acrosome reaction, and capacitation in vitro. In this study, we investigated how EVs produced by reproductive tract epithelium affect frozen-thawed bull sperm function. EVs were collected from male and female bovine reproductive tracts: Seminal plasma (SP), Estrus oviducts (EO), Diestrus oviducts (DO), and Organoid (OO) EVs. We hypothesized that seminal plasma (SP) and Diestrus (DO) EVs would inhibit sperm capacitation, and Estrus (EO) and Organoid (OO) EVs would stimulate sperm capacitation. Sperm capacitation (including an increase in plasma membrane fluidity, the phosphorylation of certain proteins and acrosomal status) was measured using flow cytometry. The capacity for sperm binding to the zona pellucida was evaluated using a perivitelline membrane assay. Sperm fertilizing potential was determined using in vitro fertilization. Results showed seminal plasma (SP) EVs reduced the acrosome reactions and membrane fluidity of sperm, compared to control cells ($p<0.05$); while EO and OO EVs increased the percentage of sperm exhibiting the acrosome reaction ($p<0.05$). EO and OO EVs did not affect sperm binding ($p>0.05$), but a trend existed for higher fertilization rates by EV-treated sperm (67 vs 76%; $p=.13$). Moreover, OO EVs increased protein phosphorylation associated with sperm capacitation. Therefore, EO and OO EVs may capacitate bull sperm effectively for IVF and increase fertilization rates. Funded by the USDA Agriculture Experiment Station.

Undergraduate Student/ BMS

20. Modulation of *Culicoides sonorensis* Microbiome in Response to Veterinary Pharmaceuticals

Colin Korte, Jonathan Rodriguez, Tyler Sherman, Phillip Shults, Christie Mayo, Jessica Metcalf, Bradley Borlee, Grace Borlee

Culicoides sonorensis is an ecologically relevant species of biting midge and a prominent vector of livestock and wildlife diseases, such as blue tongue virus, African horse sickness virus, and epizootic hemorrhagic disease virus. Although none of these viruses can be transmitted to humans, potential methods of controlling and repressing transmission are emerging areas of research. The bacterial composition of the midge midgut may influence its ability to transmit disease. Manipulating the gut microbiome of this arthropod vector may reduce the frequency at which these diseases are transmitted. To determine how the microbiome of the midgut can be altered by exposure to drugs or antibiotics commonly used in livestock veterinary medicine, lab-reared *C. sonorensis* midges were fed bloodmeals treated with either flunixin (NSAID), fenbendazole (antihelminth), dexamethasone (corticosteroid), tetracycline (antibiotic), or ceftiofur (anti-infective). Midges were surface sterilized in 70% ethanol and the bacterial composition of the midge was determined via 16S rRNA gene sequencing. Overall trends of microbial diversity between treatments were similar, except for the ceftiofur treatment, which induced decreased diversity. Midges that had ingested bloodmeal with ceftiofur had a higher relative abundance of *Aeromonas spp.* and *Morganella spp.* as compared to the other treatments. The ceftiofur bloodmeal-treated midges had a decreased relative abundance of other notable bacteria such as *Acinetobacter*. Other drug or antibiotic treatments resulted in less dramatic changes in diversity with the most fluctuations of relative abundance being in the *Acinetobacter spp*. Future studies will investigate how these changes in the microbiome alter the dynamics of blue tongue virus replication and transmission. Funded by a CVMBS experiential learning grant award.

Undergraduate Student/ MIP

21. Investigating the Effects of Temperature Change on Oviposition and Progeny Viability of *Aedes aegypti* and *Culex tarsalis* Mosquitoes

Olivia M. Martinez, Shelby Cagle, Emma K. Harris, Rebekah C. Kading

Temperature is known to affect the transmission efficiency of mosquito-borne viruses, particularly those spread by *Aedes aegypti* (*Ae. aeqygpti*) and *Culex tarsalis* (*Cx. tarsalis*) mosquitoes. With climate change a subject of increasing concern, investigating how environmental changes impact *Aedes aegypti* and *Culex tarsalis* fecundity will inform future action for vector control and subsequent disease mitigation. Our preliminary data has shown impaired egg deposition when Rift Valley fever virus- adult *Ae. aegypti* mosquitoes were exposed to temperatures varying from typical environmental conditions. Therefore, we are investigating the relationship between altered temperatures, oviposition rates, and progeny viability within uninfected blood-fed *Ae. aegypti* and *Cx. tarsalis* mosquitoes. We hypothesize that temperature variation will negatively impact egg viability, deposition rates, and offspring development. Blood-fed female mosquitoes (n=50) will be housed individually at lower (18°C), standard (28°C), or higher (32°C) rearing temperatures. Egg production will be assessed by quantifying deposited eggs in comparison to withheld eggs, obtained by ovarian dissection. Deposited egg hatch rates will be recorded to determine offspring viability. Data collection is ongoing, but preliminary data showed increased developmental rates in *Ae. aegypti* at 32°C when compared to standard temperature. In contrast, *Culex tarsalis* mosquitoes showed impaired egg laying behavior and developmental rates at 32°C. Understanding the relationship between mosquito fecundity and temperature is of great importance for anticipating infectious disease dynamics in a complex and shifting global environment. MARC Scholar Funded by a grant from the National Institute of General Medical Sciences of the National Institutes of Health: T34GM140958.

Undergraduate Student/ MIP

22. Extraction of photolumazine with MR1-restricted T cell activity from an environmental bacterium isolated from the soil

Dayton McGrail, Carolina Mehaffy, Nurudeen Oketade, Erin Kirby, David Lewinsohn, Karen Dobos

Photolumazines (PLs) are found in all types of organisms and are mainly known for their fluorescent nature and bioactivity. Structurally, they are a type of pteridine with carbonyl groups at both the C-2 and C-4 positions. Based off of previous research, we hypothesized that a bacterium that produced pigment may also produce photolumazines. We extracted a photolumazine from a pigment-producing environmental bacterium and grew it in an isolation broth. By following previous work in photolumazine isolation, we used charcoal, vacuum filtration, centrifugal filtration, and a solid phase extraction to separate out the photolumazine from the rest of the compounds. By using thin layer chromatography (TLC), we were able to identify which samples had bioactivity and which did not. We also tested the photolumazine for MR1-restricted T cell activity. MR1-restricted T cells are activated upon response to intermediate metabolites from the riboflavin pathway and photolumazines are byproduct from these intermediates. The MR1-restricted T cells had high activity, triggered by the extracted compound. These results signify the potential usage of photolumazines as vaccine targets and/or strategies.

Undergraduate Student/ MIP

23. The use of glycopeptidolipids as a diagnostic biomarker to detect non-tuberculosis infections in Cystic fibrosis patients.

Katie Moore, Jordan Manzer, Delphi Chatterjee, Antia Amin

Non-tuberculous mycobacteria (NTM) produce glycopeptidolipids (GPLs), a class of glycolipids, which are responsible for the differentiation between rough (R) and smooth (S) colony morphologies, along with other virulence factors. Smooth colony morphotypes typically produce higher amounts of GPLs than their rough colony counterparts. Additionally, it has been found that GPLs modify in response to changing environments and that carbon starvation induces smooth colony phenotypes. Among these NTM species, *Mycobacteria abscessus* is an emerging, fast-growing pathogen that causes diverse clinical manifestations ranging from cutaneous to pulmonary infections, especially in patients diagnosed with Cystic fibrosis. *M. absc* infections require therapeutic cocktails and are often associated with treatment failures, which makes early identification paramount. The objective here is to determine whether GPLs can serve as a molecular biomarker for NTM infections. Fundamentally, this research is looking for a minimally invasive, non-culture-based assay for early identification of non-tuberculosis mycobacterial infections. From a culture of *M. absc* 390R and S, the lipids were harvested and subjected to several Folch washes. The purified lipids were then run on a Thin Layer Chromatography (TLC) plate, which allowed for the identification and extraction of the GPLs. The GPLs were then further purified and used to spike urine samples received from Cystic fibrosis patients at National Jewish Hospital. These samples then underwent alditol acetate derivatization and, subsequently, Gas Chromatography (GC) analyzation. The GC report was then compared to another for *Mycobacterium avium*, a different NTM species. It was found that these species exhibited differing glycosylation patterns for their respective GPLs, which means that GPLs can be used as a basis for identification. Ultimately, this finding supports that this methodology is successful in differentiating between NTM species within a clinical setting. This research was funded through the Cystic Fibrosis Foundation.

Undergraduate Student/ MIP

24. An in-vivo exploration of the impact of the IKK Kinase complex on prion-induced inflammation

Genova L. Mumford, Arielle Hay, Jifeng Bian, Mark Zabel, Katriana Popichak, Ronald Tjalkens, Julie Moreno

Prion diseases are rare, fatal neurodegenerative disorders characterized by the aggregation of misfolded prion protein in the central nervous system, whose transmissible agent causes conformational changes in the normal prion protein. This accumulation of toxic misfolded proteins causes inflammation and spongiform changes in the nervous tissue. There is currently no cure for prion diseases, necessitating greater understanding of cellular signaling events activated during disease to mitigate pathogenesis. The IKK kinase complex is the foundation of the NF-κβ cascade, which enables signal cascades causing the upregulation of pro-inflammatory genes in microglia and astrocytes. The microglial are the immune cell of the brain and are thought to be protective when activated to clear the brain from toxic aggregated proteins. Prion diseases have been shown to induce a NF-κβ pro-inflammatory response, thus our research explores the relationship between this inflammatory pathway and prion pathogenesis. We hypothesized that if mice lack the IKK complex (IKK KO) in the microglia prion diseases mice will accelerate disease as microglia will not have the ability to protect the brain from prion accumulation. To address this hypothesis, we compared the pathogenesis between prion inoculated in 1.) experimental IKK KO mice and 2.) wild-type controls. Mice were monitored for clinical signs throughout, and histological analysis was performed, quantifying the degree of neuroinflammation present. Our results show that IKK KO prion infected mice have a significant increase in glia numbers and changes in astrocyte morphology compared to wild-type controls. Additionally, IKK KO prion mice have a reduction in neurons present in the CA1 region of the hippocampus and presented with a more rapid onset of clinical signs, succumbing to disease significantly earlier. Thus, our data supports that the microglial IKK kinase complex is essential to protect the brain from prion induced pathogenesis.

Undergraduate Student/ ERHS

25. Susceptibility of Jamaican fruit bats wing punch cells to Cedar virus

Luke Namesnik, Phillida A. Charley and Tony Schountz

Bats are reservoir hosts of a variety of RNA viruses, including paramyxoviruses. Cedar virus (CedPV), a negative sense RNA virus in the *Paramyxoviridae* family, was isolated from pteropid bats and is closely related to Hendra virus (HeV) and Nipah virus (NiV). However, CedPV is not known to cause disease, unlike NiV and HeV, which causes lethal infections in humans. The purpose of this study was to determine if Jamaican fruit bats (*Artibeus jamaicensis*) wing punch cells (AJWP) were susceptible to an infectious clone of CedPV. The AJWP were inoculated at different MOIs of 0.01, 0.1 and 1. Media were collected at various time points and photos were taken using the Revolve ECHO microscopy. The results show that AJWP cells were susceptible at all MOIs but only the MOI of 1 produced infectious virus. Cells showed signs of CPE, principally syncytia, which is common among paramyxoviruses. The data suggests that a primary bat wing punch cell line can be maintained and can produce infectious virus. Future direction for the experiment involves separating different cell types in the AJWP to determine what factors may be affecting the production of infectious CedPV in the cell line.

Undergraduate Student/ MIP

26. Inhaled agricultural dust exposure protects against secondary *Streptococcus pneumoniae* infection.

Morgan L. Pauly, Logan S. Dean, Alissa Threatt, Maelis Wahl, Kaylee Jones, Malea Barahona, Emmanuel Oyowele & Tara M. Nordgren

Repeated inhalation exposure to agricultural dust creates a chronic inflammatory environment within the lungs. This exposure causes severe pathologic damage within the lungs, characterized by decreased alveolar space, perivascular and bronchiolar inflammation, and the development of immune cell aggregations. Given the severity in lung pathology of agricultural dust exposure (ADE), we were interested in how the lung would handle a secondary exposure to *Streptococcus pneumoniae* (*S. pneumo*), the most common cause of community acquired pneumonia. We pre-exposed male and female C57BL/6 mice 14 times intranasally with either dust extract (DE) or a PBS vehicle control. Following dust installations, a single installation of 10^7 colony forming units (CFU) of S. pneumo, or PBS vehicle control was given. Mice were sacrificed 72 hours post infection and CFU was determined in the spleen, lungs, and airway (via bronchoalveolar lavage). We found that all mice who had been chronically exposed to dust prior to bacterial exposure were able to clear the infection entirely, while vehicle control pretreatments demonstrated a recoverable bacterial burden in the lung, spleen, and airway. The recoverable CFU within the spleen indicates that the infection had gone systemic. We hypothesize that this clearance of bacteria within the DE pretreatment is due to a "priming" effect on the mouses adaptive immune system, suggesting that ADE may not increase S. pneumo induced lung infection. Funding for this project was provided through the NIH/NHLBI R01HL185926

Undergraduate Student/ ERHS

27. Establishing molecular mechanisms of glial-mediated response in a chronic pain *in vitro* model

Emily M. Perkins, Elizabeth A. Ninke, Mark D. Zabel, Julie A. Moreno, Katriana A. Popichak

More than 1 in 5 adults in America experience chronic pain (CP), often concomitant with other health conditions ranging from cancer to fibromyalgia, to rheumatoid arthritis. Although the understanding of pathological pain is improving, many findings revolve around neuronal mechanisms. Recently, non-neuronal cells, glia, such as microglia, the macrophage of the brain, have emerged as key players within pathological and CP mechanisms demanding further examination. Gliosis or glial activation, characterized by neuroinflammation and activation of the transcription factor, NF-kB, accompanies chronic pain recapitulated in *in vivo* models, however, a better-defined *in vitro* model of CP is warranted to aid in further interrogation into the key cellular mechanisms associated with CP. Thus, we hypothesize that *treating murine glial cells with Complete Freund's Adjuvant (CFA) will establish a novel in vitro model of CP, in which we will characterize neuroinflammatory response and NF-κB-dependent signaling like that seen in well-established in vivo models of CP*. To test this hypothesis, we propose to measure inflammatory expression in RAW 264.7 cells, a macrophage cell line, as well as primary glia that have been treated with CFA. Here, we measured cell viability and proinflammatory gene expression. Additionally, we will expose primary neurons to glia conditioned media from glial and RAW cell experiments and measure neuronal viability to determine the role that NF-kB mediates in glial-derived neuroinflammatory factors released into their environment. Taken together, these data will reveal the importance of glial and macrophage cell response and cellular mechanisms in the elucidation of potential therapeutic targets to inhibit chronic pain involved in a multitude of debilitating disorders.

Undergraduate Student/ MIP

28. Glial inflammatory response to metronidazole exposure in a murine, *in vitro* model

Quinn S. Pogge, Elizabeth A. Ninke, Emily M. Perkins, Julie A. Morano, Mark D. Zabel, Katriana A. Popichak

Nitroimidazoles are a group of antimicrobials that target anaerobic and microaerophilic bacterial infections alongside protozoal infections. One of the most prescribed nitroimidazoles, metronidazole (MTZ), is prescribed for bacterial infections in the gastrointestinal and urinary tract, heart, bones, lungs, blood, and nervous system. High dose usage of MTZ is associated with certain neurotoxic features in humans, including reversible memory loss, although the exact cause is unknown. Since MTZ's mechanism of action is inhibition of DNA synthesis, this may lead to the targeting of other cells such as neuronal and non-neuronal brain cells, glia, suggesting that unintended consequences of increased pro-inflammatory response could occur. *Therefore, we hypothesize that metronidazole exposure to primary murine glial cells results in neurotoxic proinflammatory gene expression*. To test our hypothesis, we utilized a primary, murine in vitro model to measure glial response and whether glial-mediated cytokine release alters neuronal viability. Measuring inflammatory cytokines and neuronal exposure to glial-conditioned media lends insight in better understanding glial-neuronal signaling response to MTZ, further elucidating mechanisms behind MTZ neurotoxicity. Funding for this project was provided through the Microbiology, Immunology and Pathology department of Colorado State University.

Undergraduate Student/ MIP

29. Cheating death by gambling with chromosomes: karyotypic variation drives the emergence of virulent traits in an opportunistic fungal pathogen

Isabella F. Prosceno and Jacob D. Diaz, and Lydia R. Heasley

How do opportunistic fungi leverage their phenotypic potential to survive in the changing environment of the host? Many canonical adaptation strategies rely on conserved environmental response pathways, which allow a cell to first sense an environmental change, and then induce an appropriate phenotypic response. But cells can also stochastically toggle between phenotypic states, even without a cue from their surroundings. This latter behavior, known as phenotype switching, is thought to function as a bet hedging strategy to boost the adaptive potential of a population by enabling individuals to rapidly and dynamically sample new phenotypic space. This diversification process has been repeatedly observed amongst clonal populations of many opportunistic microbes. In fact, decades of observation suggest that phenotype switching may be one of the few conserved mechanisms used by opportunistic species across the tree of life to generate phenotypic novelty. Our group recently discovered that stochastic alterations in karyotype, driven primarily by whole chromosome gains and losses (i.e.., aneuploidy), represented a penetrant mechanism by which an opportunistic isolate of the baker's yeast *Saccharomyces cerevisiae*, a strain called *YJM311*, switched phenotype. In the present study, we set out to determine whether the karyotypic variants which arose spontaneously within populations of *YJM311* displayed differential sensitivities to the anti-fungal agent fluconazole, a key trait associated with virulence and opportunistic potential.

Undergraduate Student/ Other

30. Tibialis Anterior Maximal Force Capacity and Balance Assessment Scores in People with Multiple Sclerosis

Matthew W. Scarsbrook, Chris M. Patrick, & Brett W. Fling

Multiple sclerosis (MS) is a chronic neurodegenerative disease that results in damage to the central nervous system and hinders communication between neurons. Impaired neuronal communication reduces the amount and quality of sensory input from the periphery and decreases force capacity, which may lead to gait and balance impairments. These manifestations put people with multiple sclerosis (PwMS) at an increased risk of falls and other adverse events. Clinical balance evaluations such as the Mini Balance Evaluation Systems Test (Mini-BEST) can be used to evaluate the degree of balance and mobility deficits that PwMS experience. Components of the Mini-BEST include 14 assessments that target 4 balance systems: anticipatory postural adjustments, reactive postural responses, sensory orientation, and dynamic gait. The capacity for quick and powerful force output from the muscles of the lower body is critical for the function of all these balance systems and for reducing the likelihood of falls. Understanding how force capacity influences performance on each MiniBEST subcomponent can inform rehabilitative efforts. My project compares maximum force output of the tibialis anterior muscle of the lower leg with participants scores for all 4 subcategories of the MiniBEST. Results from this study will emphasize the need for future studies to look at more specific areas of force generation in PwMS so novel rehabilitation methods aimed at reducing falls and other injuries can be created. Funded by the Charles A. Dana Foundation, David Mahoney Neuroimaging Program.

Undergraduate Student/ Other

31. An integrated analysis of the transcriptomic effects observed following wildland firefighter relevant occupational exposures in the pre-frontal cortex of male mice

Emma J. Smith, Adam J. Schuller, Luke B. Montrose

Wildfires continue to increase in frequency and intensity following trends of climate change across the globe. At the forefront of these extreme weather events are wildland firefighters (WLFF) who are exposed to several occupational factors (e.g., wildfire smoke, poor nutrition, sleep deprivation, etc.). While it is known that these exposures are associated with adverse central nervous system (CNS) health outcomes separately, little is known about the effects of combined exposures which WLFFs are subjected to. To address this, we examined the literature for bulk RNAseq datasets which originated from studies assessing WLFF-relevant exposures in CNS tissue. We identified 3 factors (wildfire smoke particulate matter, high fat diet, and psychological stress) with similar study populations (male mouse pre-frontal cortex) and performed an integrated analysis of the 8,367 differentially expressed genes (DEGs) as reported in each parent study. We found that 117 DEGs overlap between the three studies (6 consistently up, 13 consistently down, and 98 with directional discordance) which we further examined for functional enrichment and found significant differences in pathways relevant to synaptic transmission, specifically release cycles for common neurotransmitters. Additionally, protein-protein interaction network analysis revealed proteins with a central hub made up of proteins highly involved in axonal regulation. This is the first attempt to characterize molecular changes that are associated with multiple relevant exposures in WLFF by mining mouse model data. The results reported here motivate subsequent prospective studies of these factors using multi-hit mouse models to tease out potential synergistic mechanisms in the context of WLFF occupational health effects.

Undergraduate Student/ ERHS

32. Characterization of Gene Expression in Increased Omega-3 Fatty Acid Model Following Organic Dust Exposure

Maelis J. Wahl, Logan S. Dean, Tara M. Nordgren

Chronic inhalational exposure to organic dust (OD) has been demonstrated to cause inflammation within the respiratory tract, leading to both acute and chronic respiratory disease. The utility and efficacy of omega-3 fatty acids (n-3 FA) to resolve the OD-induced inflammatory environment is under active investigation. Via a transgenic mouse model of increased omega-3 fatty acids, the *Fat1* mouse allows for n-3 FA investigations without the need for traditional dietary interventions. Previous investigations have revealed the *Fat1* mouse decreases inflammatory signatures and lung pathology following chronic organic dust exposure (ODE), however, the genotypic drivers behind this resolution have not been explored. Using an established model of ODE, we collected the left lung from C57BL/6 (WT) and *Fat1* mice exposed to 14 intranasal installations of dust extract in PBS (Phosphate Buffered Saline) or a PBS vehicle control. RNA was extracted via Trizol and sent to the Colorado State University Veterinary Diagnostic Lab for quantification and quality control. All samples passed QC and were run on the Mouse Myeloid and Mouse Immunology targeted RNA sequencing platform by Nanostring. Gene expression was analyzed in Nanostring's nSolver software and visualized in ROSALIND. The myeloid and immunology panels comparing WT and *Fat1* samples, with or without dust exposure, revealed that ODE samples had a significantly higher upregulation of transcripts compared to their saline counterparts. Inflammatory and innate immune response related transcripts were demonstrated most commonly in the myeloid panels of both genotypes, while phagocytic and inflammatory transcripts were more frequent in the immunology-based panel of both genotypes. Given the transcript-level differences noted in the *Fat1* model, future investigations will work to investigate the underlying mechanisms of these transcripts in promoting resolution following dust exposure and treatment with n-3 FAs. Research funded by NIH/NHLBI R01HL185926.

Undergraduate Student/ ERHS

33. Prevention of inflammation and loss of myelin in a mouse model of Multiple Sclerosis using Nanoligomers™

Grace Weisman, Sydney Risen, Sadhana Sharma, Prashant Nagpal, Anushree Chatterjee, Vincenzo Gilberto, and Julie Moreno

Multiple Sclerosis (MS), a leading cause for neurological damage among young adults, is the most common inflammatory and demyelinating disease of the central nervous system It is estimated that nearly 2.8 million people worldwide are affected by Multiple Sclerosis. Although there is no cure for MS, treatment currently focuses on limiting prevalence, duration of attacks, reducing clinical relapses, managing MS symptoms, and slowing the progression of the disease. NLRP-3 and NF-**κ**B are two key inflammatory signaling molecules known to be upregulated in MS and other neurogenerative diseases. Sachi Bioworks has developed Nanoligomers™ that inhibit both NLRP-3 and NF-**κ**B and we hypothesize that inhibition of these neuroinflammatory pathways will be neuroprotective in a mouse model of MS. To address this hypothesis, we used the Experimental Autoimmune Encephalomyelitis (EAE) murine model, a widely used model for MS. Twenty-four induced EAE female mice aged 11-13 weeks were divided into four treatment groups. The four groups included mice with no EAE, EAE and vehicle, EAE with SB_N1_111 (NF-**κ**B and TNFα inhibitor), and EAE and SB_NI_112 (NF-**κ**B and NLRP3 inhibitor). Nanoligomers™ treatment administration occurred three times a week via intraperitoneal injections at a dose of 150 mg/kg. Daily monitoring was conducted to assess the appearance and progression of clinical signs. We found that the translational inhibition of NLRP-3 and NF-**κ**B significantly prevented the onset of clinical symptoms in the EAE mouse model throughout the disease course. Additionally, the inhibition of these pathways decreased significantly both microglial activation and immune cell infiltration of the spinal cord using both Iba1 immunohistochemistry for macrophage infiltration and Luxol Fast Blue staining for myelin integrity. To conclude we were able to identify that the inhibition of two pro-inflammatory pathways simultaneously in mouse model of MS is clinically protective by reducing inflammation in spinal cord allowing neuronal myelin integrity to say intact. Research funded by NASA SBIR 80NSSC22CA116, Sachi Bio, and CVMBS Murphy Turney Fund at Colorado State University

Undergraduate Student/ ERHS

34. Neuropathological brain changes in a Dunkin-Hartley Guinea Pig Model as a naturally occurring model of Alzheimer's Disease

Kristin Weninger, Amanda S. Latham, Chase Gross, Isla K. Anderson, Karyn L. Hamilton , Kelly Santangelo, and Julie A. Moreno

Alzheimer's Disease (AD) and dementia impact approximately 50 million people worldwide, and the research investigating these diseases has been rapidly evolving. Current studies of neurodegenerative disease and aging processes use genetically engineered mice or human derived stem cells. However, these approaches do not account for natural forms of aging and have been shown to not directly translate to human disease. This study examined the behavior and neuropathology of young (5 months old) and aged (15 months old) outbred guinea pigs. Two strains were examined, the Dunkin Hartley (DH) strain, which displays enhanced age-related decline, and the pigmented guinea pig (PET), which demonstrates delayed progression of age-associated neuropathology. Postmortem brain tissue samples were stained with immunofluorescence to highlight aging brain biomarkers. Glial inflammation, including astrocytes and microglia, and misfolded protein pathologies were analyzed among young and aged guinea pigs. A significant increase in activated astrocytes and hyperphosphorylation of tau, along with a decrease in neurons, were observed in the hippocampus of DH animals, which closely resembles pathologies observed in AD. Through this data, we establish that the Dunkin Hartley guinea pig may be an appropriate rodent model as it is the first rodent model to be naturally aging and experiencing brain pathology similar to AD and other AD related diseases (ADRDs).

Undergraduate Student/ ERHS

35. Characterization of modes and kinetics in mutation accumulation models in *Saccharomyces cerevisiae*

Mackenzie Wienke, Joseph Stewart, Camryn Schmelzer, Juan Lucas Argueso

Gradualism is the primary and most supported mode of mutation accumulation, in biology, where mutations steadily accumulate throughout an organism's lifetime and its species evolution. An additional model of mutation accumulation that has been observed in several cell types, such as heterogenous tumors, is punctuated equilibrium (i.e., bursts) where multiple mutations can accumulate within a few cell cycles during periods of systemic genomic instability (SGI) before returning to a period of stability. We use loss of heterozygosity (LOH) tracts to model mutation accumulation events which are caused by allelic interhomolog mitotic recombination events that result in regions of heterozygosity that become homozygous. We hypothesize that mutation bursts can arise within a single-cell lineage and that these events are not as rare as previously thought. To test our hypothesis, we constructed a hybrid yeast diploid containing ~55,000 heterozygous single nucleotide polymorphisms (SNPs) along with two gene reporter cassettes, ADE1-CAN1 and ADE2-kIURA3 inserted near the end of chromosomes 13 and 15, respectively. Phylogenetic experiments were used to infer the timing of these burst events. When an LOH event occurred at either of the cassettes, red pigment adenine precursors buildup resulting in a red colony. Red colonies on plates containing phylogenies of up to 6 cell divisions were whole genome sequenced (WGS) to search for additional LOH tracts. When additional LOH tracts were found, the rest of the colonies on the corresponding plate were PCR-genotyped via restriction fragment length polymorphism (RFLP). Our results showed that bursts do indeed arise in single-cell lineages as multiple, independent LOH tracts accumulated within one to two cell divisions. Through the screening of over 5,000 single-cell lineages, we have been able to classify phylogenies with both gradual and burst events, determining the relative frequency of each mutation accumulation mode.

Undergraduate Student/ ERHS

36. Ketamine's rapid antidepressant effects are mediated by Ca^{2+}-permeable AMPA receptors

McKennon J Wiles, Evelina Bouckova, Madison H Wustrau, Anastasiya Zaytseva, Isabella G Schmidt, Hadassah Mendez-Vazquez, Seonil Kim

Ketamine is shown to enhance excitatory synaptic drive in multiple brain areas, which is presumed to underlie its rapid antidepressant effects. Moreover, ketamine's therapeutic actions are likely mediated by enhancing neuronal Ca^{2+} signaling. However, ketamine is a noncompetitive NMDA receptor (NMDAR) antagonist that reduces excitatory synaptic transmission and postsynaptic Ca^{2+} signaling. Thus, it is a puzzling question how ketamine enhances glutamatergic and Ca^{2+} activity in neurons to induce rapid antidepressant effects while blocking NMDARs in the hippocampus. Here, we find that ketamine treatment in cultured mouse hippocampal neurons significantly reduces Ca^{2+} and calcineurin activity to elevate AMPA receptor (AMPAR) subunit GluA1 phosphorylation. This phosphorylation ultimately leads to the expression of Ca^{2+}-Permeable, GluA2-lacking, and GluA1-containing AMPARs (CP-AMPARs). The ketamine-induced expression of CP-AMPARs enhances glutamatergic activity and glutamate receptor plasticity in cultured hippocampal neurons. Moreover, when a sub-anesthetic dose of ketamine is given to mice, it increases synaptic GluA1 levels, but not GluA2, and GluA1 phosphorylation in the hippocampus within 1 hr after treatment. These changes are likely mediated by ketamine-induced reduction of calcineurin activity in the hippocampus. Using the open field and tail suspension tests, we demonstrate that a low dose of ketamine rapidly reduces anxiety-like and depression-like behaviors in both male and female mice. However, when *in vivo* treatment of a CP-AMPAR antagonist abolishes the ketamine's effects on animals' behaviors. We thus discover that ketamine at the low dose promotes the expression of CP-AMPARs via reduction of calcineurin activity, which in turn enhances synaptic strength to induce rapid antidepressant actions.

Undergraduate Student/ BMS

O-1. Is the Occurrence of Antimicrobial Resistance Genes Higher on Chromosomes Compared to Plasmids among *Salmonella Typhimurium* Isolates from Cattle?

Sami Ullah Khan Bahadur, Nora Jean Nealon, Roberta Magnuson, Mo Salman, Joy Scaria, Sangeeta Rao

Understanding resistance genomics of *Salmonella Typhimurium* is a crucial aspect of antimicrobial resistance (AMR) due to potential transmission of resistance genes among humans and livestock, including cattle. The existence of class-I Integrons, a crucial mobile gene platform, in variants of Salmonella species is linked to distinct virulence factors that are absent in variants lacking these integrons. Moreover, many studies have associated integron size with AMR patterns, but not determined the location of these integrons on the bacterial genome. Hence, this study aims to use bioinformatics tools to identify the location of integrons: Chromosome vs. Plasmids. Thirty-three *Salmonella Typhimurium* isolates of bovine origin were collected from various laboratory repositories across the United States, which were subjected to antimicrobial susceptibility testing. Integron sizes were identified through PCR and gel electrophoresis. Whole-genome and integron sequencing was performed using an Illumina MiSeq, followed by denovo genome reconstruction in SPAdes through a Geneious Prime interface. Plasmid sequences for each sample were identified and separated from whole genome sequences using Plasmid finder. AMR genes and virulence genes were identified through BLAST searches against MEGARes2 Database and Virulence Factor Database, respectively. These gene sequences from each isolate were identified on corresponding integrons and blasted with plasmid and chromosomal sequences to find the exact location. Among the 33 isolates, 60% were ACSSuT (Ampicillin, Chloramphenicol, Streptomycin, Sulfa, Tetracycline) type multi-drug resistant, and 48.4% of isolates carried 1000 and 1200 bp integrons. Overall, floR followed by qacEdelta1 and sul1 AMR genes and type III secretion system (spv) and fimbriae (pef) virulence genes were identified among these isolates, majorly representing MDR. With the ongoing results, this study will help us to understand location of class-I Integrons harboring virulence and resistant genes on genome with a goal to contribute to knowledge of the dynamics of gene transfer and evolution within bacterial populations.

Graduate Student/ CS

O-2. Immunomodulatory effects of articular chondroprogenitor cells

Taryn Boxleitner, Lynn Pezzanite, Lyndah Chow, Parvathy Thampi, Steve Dow, Brian Johnstone, and Laurie Goodrich

Osteoarthritis (OA) is a degenerative disease that results in destruction of articular cartilage in joints of many species. OA has an inflammatory component that provides a potential therapeutic target for slowing OA progression. Bone marrow-derived mesenchymal stromal cells (MSCs) may be beneficial in the context of OA due to their immunomodulatory activity. However, they do not appear to stimulate new cartilage growth when injected into joints and present challenges in clinical use due to variability in donors and preparations. Articular chondroprogenitors (ACPs), isolated from healthy articular cartilage, pose advantages as an alternative therapy. Unlike MSCs, they have the ability to divide and expand in culture long-term without loss of chondrogenic capability. The goal of this study was to compare functional activity of equine ACPs and MSCs *in vitro*. We hypothesized that ACPs have comparable immunomodulatory properties to MSCs. Equine ACP clones and MSCs from three donors were grown to generate conditioned medium (CM) which was then used to determine the effects of ACP and MSC secretory products on macrophages. The macrophages were activated using a combination of IL-1β and TNF-α, and cultured in 1:1 media to CM. Their secretome profile was then analyzed with an equine multiplex cytokine assay. The macrophages exposed to ACP or MSC CM produced significantly less inflammatory cytokines (IL-1β ($p<0.0001$) and TNF-α ($p=0.01$, $p=0.03$)) compared with positive controls. The macrophages cultured with ACP CM produced significantly more anti-inflammatory IL-10 versus untreated controls ($p<0.01$). We concluded that ACPs exhibit immunomodulatory functions and further investigation of ACPs as a therapy for OA is warranted. Research funded by the Hong Kong Jockey Club Equine Welfare Research Foundation. Student support provided by NIH Training grant T35OD015130.

DVM Student/CS

O-3. Behavioral strategies of state animal health officials on national disease reporting

Matthew Bradley, Quinn Treuting, Sangeeta Rao

National disease reporting is one of the key responsibilities of State Animal Health Officials (SAHOs). They encounter multiple barriers that inhibit their ability to submit timely, accurate, and complete disease reports. In order to address these barriers, seven SAHO's were interviewed using a semi-structured method, to obtain their perspective on current behavioral barriers of reporting in their respective regions and the incentives or strategies that could be implemented to improve disease reporting. Four regions were identified, those being Midwest, West, Northeast, and Southeast, to identify commonalities and differences in current reporting strategies among these regions. Several SAHOs experienced positive experiences working with their state laboratories, communicating with the USDA, and with their state's veterinarians, however not all experiences were positive. Several SAHOs expressed common concerns in the West, Northeast, and Southeast regions over timely, accurate, and complete reporting issues due to communication complications between veterinarians and the state due to a lack of understanding of how and when to report reportable disease. Additional concerns included time constraints, difficulty navigating current disease reporting software, a lack of uniformity of what diseases to report, and privacy of submitted information. Notably, the Midwest region did not experience the same barriers, but instead had positive experiences among the current approaches. Additionally, SAHOs were asked to identify groups to train and provide supplemental educational opportunities to help improve current strategies. These group identified were veterinarians as the primary group to train, with laboratories, academia, and epidemiologists being secondary groups. SAHOs were also asked their opinions on incentivizing veterinarians to report, but majority of SAHOs indicated that it is a veterinarian's job and incentivizing would be unnecessary. SAHOs did indicate, however, that further training with continued education credits would be beneficial. Research support provided by USDA-APHIS.

Graduate Student/ CS

O-4. Extracellular vesicles as a potential mechanism of hematogenous transport of Chronic Wasting Disease

Jesse Cole, Erin McNulty, Audrey Sandoval, Amy Nalls, Joseph Westrich, Candace Mathiason

Chronic Wasting Disease (CWD) is a rapidly spreading, fatal neurodegenerative or prion disease of cervid species (deer, elk, moose and reindeer). CWD is the most efficiently transmitted of all the prion diseases and is currently detected in captive and free-ranging cervid populations in 30 U.S. States, 4 Canadian Provinces, Europe, and Asia. The effective transfer of CWD among cervids has been largely attributed to horizontal transmission by direct animal-to-animal contact via exchange of bodily secretions (saliva, blood, urine and feces), and by indirect contact with the infectious agent shed in these products to the environment. Prions have been detected in blood, as well as within the pregnancy microenvironment and fetal tissues harvested from CWD-infected cervids. To further investigate CWD peripheralization mechanisms and how prions traffic across the placental barrier, we are assessing the role blood serum-derived extracellular vesicles may play in these processes. Here, EVs were isolated from blood serum collected from experimentally CWD-infected white-tailed deer. Nanoparticle tracking analysis (NTA) was performed to quantify the size distribution and concentration of EV isolates. We are further assessing EV isolates for the presence of prions by western blot (PrPSc) and real-time quaking induced conversion (RT-QuIC) (amyloid seeding activity). These studies will provide the basis for continued studies determining CWD peripheralization in the host, and permit further investigation of EVs as a potential biomarker for CWD diagnostic testing. Research support provided by NIH-NIAID R01AI112956. Student support provided by NIH MSTP Training Grant T32GM136628.

DVM/PhD Student/MIP

O-5. Optimization of bluetongue virus RNA extraction and next-generation sequencing protocols for enhancement of disease surveillance

Tillie Dunham, Tyler Sherman, Justin Lee, William Wilson, Lee Cohnstaedt, Tavis Anderson, Kirsten Reed, Mark Stenglein, & Christie Mayo

Bluetongue virus (BTV) is an economically important arbovirus of domestic and wild ruminants. Transmitted by *Culicoides* biting midges, BTV is an orbivirus with a double-stranded RNA (dsRNA) genome consisting of 10 linear segments. Segment 2, which encodes for the viral protein that induces the serological response in the mammalian host, determines the serotype of the virus. To date, there are over 29 BTV serotypes recognized. As a segmented virus, BTV can undergo rapid evolution through mutation and reassortment of individual segments between coinfecting parental serotypes. Sequencing of all ten genomic segments for BTV isolates will enhance estimation of BTV diversity as serotype determination relies solely on a single genomic segment. While whole genome sequencing permits investigation of genetic diversity, there are inherent challenges posed by dsRNA viruses. Current protocols for sequencing of BTV require refinement to improve efficiency. Our objective was to optimize RNA extraction and next-generation sequencing workflows to facilitate the analysis of a large historical collection of BTV isolates. This was accomplished by evaluating the nucleic acid concentrations, molarity, coverage, and alignment to the BTV genome outcomes of different protocols. Variations in extraction protocols included automated extraction, manual extraction, usage of carrier RNA, applications of different DNAse treatments, and performing LiCl treatments. Variations in library preparation included employment of different preparation kits, fragmentation techniques and number of amplification cycles. Results indicated that automated KingFisher extraction using carrier RNA and turbo DNAse was the most effective extraction method. For library preparation, lower fragmentation temperatures and increased library amplification cycles resulted in more reads that aligned with BTV. This optimization study is beneficial for improving the workflow for sequencing numerous BTV isolates and will enhance the crucial efforts to improve the surveillance of this important disease. This work was funded by the USDA-NIFA AFRI grant number 2019-67015-28982 as part of the joint USDA-NSF-NIH-BBSRC-BSF Ecology and Evolution of Infectious Diseases program as well as ARS-NACA Collaboration No. 58-3022-2-035.

Graduate Student/ MIP

O-6. Characterizing the Circadian Rhythms of PrP-Associated Transgenic Mice

Kaitlyn Forrest, Erin McNulty, Joesph Westrich, Candace Mathiason

Circadian rhythms regulate approximately half of the mammalian genome and therefore are an important factor to investigate when interrogating gene function. The overall goal of this study aims to characterize the molecular and cellular circadian rhythms in transgenic mice associated with the prion protein (PrP; encoded by the Prnp gene). Brain tissue and lymphoid-associated immune cells harvested from FVB-KO (Prnp knockout) mice were assessed, respectively, for Prnp and circadian gene expression by qPCR in comparison to C57bl/6j (wildtype) and Tg(CerPrP-E226)5037$^{+/-}$ (Prnp over-expressing, herein referred to as 5037) mice or by flow cytometry to evaluate cellular population proportions (CD45,CD3,CD4,CD8a,NKp46,B220,MHC-II,F4/80,CD11b). FVB-KO-derived bone marrow cells were seeded and treated with 200nM dexamethasone to induce circadian synchronization and stained for surface markers (CD45,CD41,CD117) and intracellular proliferation marker Ki67. C57bl/6j brain gene expression was cyclic for core clock and clock-controlled genes, but Prnp expression was arrhythmic while in 5037 brain, Prnp expression is clearly rhythmic. Compared to the wildtype condition, FVB-KO and 5037 mice displayed strain-specific dyssynchronous or absent cycles of brain circadian gene expression. Immunophenotyping of FVB-KO splenic and lymph nodes revealed rhythmic and arrhythmic lymphocyte populations. B220+ cells oscillated in both organs, however CD4+ and CD8+ T-cells only oscillated in the lymph nodes. NKT cells trended towards cycling in the lymph nodes but not in the spleen, while traditional NK cells demonstrated inverse trends. Bone marrow cells from FVB-KO mice display rhythmic expression of cell surface markers and proliferation in-vitro. This study provides the first characterization of molecular and cellular rhythms in PrP-associated transgenic mice. Circadian rhythms are present with differential contexts of PrP expression; however, the maintenance is not uniform and circadian gene expression is largely dyssynchronous – if the rhythm is present. The data in this study suggests a relationship between the circadian system and native PrP.

Graduate Student/MIP

O-7. Health Effects Of Uranium Contamination In Water On The Sweetwater Chapter Of The Navajo Nation

Christian Grabowski, Gilbert John, Thomas Johnson

The Sweetwater Chapter is one of the most isolated chapters in the Navajo Nation, lacking convenient access to clean water wells for livestock. Like many of the regions on the Navajo Nation's land, uranium mining for the United States' military and other purposes was conducted throughout the 1940s-1980s. Uranium, from either mining or naturally, is found in many of the water sources located in the Sweetwater Chapter. The scarcity of clean water sources is of concern for residents, as few studies exist on the long-term health implications of uranium in water. The goal of this study is to research and inform the Sweetwater Chapter members of the health risks from uranium in water, and the risk, if any, to its members, wildlife, livestock, and the environment. This evaluation will be done through a series of various sample collection techniques to ascertain the uranium content of local water wells and springs, through both livestock and wild animals, and ultimately the impact on residents and the community. Techniques include water sample analysis from local wells, camera traps to ascertain the amount and type of animals utilizing springs (and ascertain internal animal dose), and bone sample analysis from deceased wildlife, livestock, and game animals. Student support provided by Nuclear Regulatory Commission Graduate Research Fellowship Grant Award (31310021M0028).

Graduate Student/ ERHS

O-8. Characterization of the surface-associated polysaccharides in *Burkholderia pseudomallei*

Amr Ramadan, Lily Filipowska, Kevin Martin, Grace Borlee, and Bradley Borlee

In this study, we characterized the surface-associated polysaccharides (SAPS) in *Burkholderia pseudomallei* (Bp), a prevalent saprophyte in tropical and subtropical water and soil. Bp is the causative agent of Melioidosis, a potentially fatal bacterial infection, whose global impact remains underrecognized. Recently declared endemic in the Mississippi Gulf Coast in 2022, Bp poses a significant public health hazard. We hypothesized that Bp SAPS, including Capsular Polysaccharides (CPS), Exopolysaccharides (EPS), and Lipopolysaccharides (LPS), play crucial roles in host infection, immunity evasion, biofilm formation, and are differentially expressed during infection to enable survival in diverse environments. Our methodology involved characterizing Bp SAPS biosynthetic clusters by creating a panel of polysaccharide deletion mutants via conjugative allelic exchange with pEXKm5 non-replicative vectors, then creation of verified mutants each either producing a single polysaccharide or lacking a particular polysaccharide for in-depth analysis. We assessed the role of SAPS in various phenotypes, including growth under biologically relevant infection conditions, biofilm formation, susceptibility to host components as well as antimicrobial agents, adhesion to A549 human lung epithelial cells, and infection assays in RAW246.7 macrophages. Confocal microscopy further elucidated the biofilm characteristics and SAPS' involvement in Multinucleated Giant Cell (MNGC) formation in RAW246.7 macrophages and A549 human lung epithelial cells. Notably, EPS (*becA-R*) emerged as the most critical component for biofilm formation. We also observed dynamic changes in the production of various Bp SAPS during different stages of host infection and across varied environments. Our findings offer significant insights into the multifaceted role of Bp SAPS in environmental adaptation and pathogenicity, contributing valuable knowledge to Bp environmental survival, distinctive ability to shift from a saprophytic to an aggressive intracellular pathogen, and pathogenesis. Research support provided by Ministry of Higher Education and Scientific Research - Cairo, Egypt and The Egyptian Cultural and Educational Bureau (ECEB)- Washington DC.

Graduate Student/MIP

O-9. Enhancing the antioxidant capacity of granulosa cells to mitigate the impact of heat stress

Ghyslaine G. Ramirez, Ahmed Gad, Nico G. Menjivar, Dawit Tesfaye

Heat stress (HS) adversely affects the proper ovarian function needed to facilitate the release of competent gametes for fertilization and subsequent pregnancy. Granulosa cells (GCs) provide a critical microenvironment mediating intrafollicular communication with the oocyte and their survival is critical for the development of oocyte competence. Follicular cells react to heat stress, activating heat shock proteins and the NRF2 pathway, a master regulator of cellular response to environmental stress. Phytochemicals are plant compounds such as quercetin, carnosol, and sulforaphane, found in cruciferous vegetables like broccoli and are known to induce cytoprotective effects, improving the cellular antioxidant capacity via scavenging free radicals and their interactions with lipid membranes and proteins. Here we investigate the impact of antioxidant supplementation on bovine GCs under thermal stress conditions. Initially, bovine GCs were cultured for 24 hours until sub confluency followed by quercetin (5μM), carnosol (10μM), sulforaphane (1μM), and all combined supplementation, followed by subsequent culture under normal temperature (NT) 38.5°C, and heat stress 42°C conditions for an additional 24 hours. Vehicle (DMSO) supplemented and nontreated GCs were used as controls. Cell viability assay was used to check the toxicity of the doses used for the experiments. Following harvesting, the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific™) was employed to separate nuclear and cytoplasmic protein fractions from GCs. Subsequently, the TransAM® NRF2 ELISA was applied to analyze nuclear protein extracts derived from both individual and combined antioxidant-supplementation groups under NT and HS. Supplementation of quercetin and subsequent exposure to HS has significantly increased the NRF2 protein activity compared to controls (>0.05). Moreover, even though not statistically significant, supplementation of combined antioxidants increased the activity of nuclear NRF2 protein. Subsequent phases of experimentation will investigate the expression of stress-related genes and proteins, ROS accumulation, mitochondrial membrane potential JC-1, and antioxidant capacity assay through Glutathione quantification. Research supported by USDA Agriculture Experiment Station.

Graduate Student/ BMS

O-10. Household air pollution and Inflammation: do lifestyle patterns matter?

Christian Sewor and Maggie Clark

Air pollution exposure leads to adverse health through oxidative stress and systematic inflammation. In observational studies, healthy lifestyle patterns (e.g., increased antioxidant consumption and physical activity) have reduced the health impact of air pollution. We previously reported substantial reductions in fine particulate matter (PM2.5) and black carbon (BC) following a stepped-wedge randomized intervention of the wood-burning *Justa* cookstove in rural Honduras. Within the trial, we further explored the modifying role of diet and physical activity on the association between household air pollution (HAP) exposures and C-reactive protein (CRP) levels. We recruited 230 female cooks for a 3-year study that included 6 repeated measures of exposure and health every ~6 months. We estimated exposure using daily and long-term averages of personal and kitchen PM2.5 and BC concentrations. Self-reported dietary and physical activity information was used to estimate baseline lifestyle inflammatory scores (LIS). CRP levels were assessed via dried blood spots. Linear mixed models with 3-way interactions for HAP exposure, LIS, and age were used to explore associations. LIS was positively associated with CRP (0.40 log-ng/ml [95% CI: 0.26, 0.54]). The main-effect associations between HAP exposures (per log-μg/m3) and log-CRP levels were inconsistent and mostly negative (e.g., personal PM2.5: 0.02 [95% CI: -0.02, 0.06]; personal BC: -0.03 [95% CI: -0.05, -0.01]). Effect modification results were generally weak and inconsistent. A pattern of increasing associations between personal PM2.5 and CRP was observed across increasing tertiles of LIS among those <40 years (Q1 = 0.01 [95% CI: -0.06, 0.07; Q2 = 0.02 [95% CI: -0.07, 0.12; Q3 = 0.10 [95% CI: -0.08, 0.28]) but not among those >40 years (p-interaction=0.61). Our novel investigation did not provide clear evidence of modification; future efforts are necessary to understand the potential modifying effects of inflammatory lifestyle patterns within interventions that reduce exposure to air pollution.

Graduate Student/ERHS

O-11. Spatial variation in access to alcohol, tobacco, and marijuana and association with sociodemographic characteristics in Denver, Colorado

Marshall Thomas, Sheryl Magzamen

Increased access to retail locations for alcohol, tobacco, and marijuana has been associated with increased substance use, decreased life expectancy, and increased mortality. In Colorado, retail density has been associated with a greater percentage of racial and ethnic minorities, lower median household income, and higher unemployment, indicating that access to substances is inequitable. Despite the importance of understanding the burden of legal substance retailers on communities, there has been significant methodological variation within and between substances, and few studies have considered all three simultaneously. We address this gap in Denver by describing access to alcohol, tobacco, and marijuana in Denver using robust and standardized techniques, and assess how access to these retailers varies by sociodemographic characteristics. Retail licenses for alcohol, tobacco, and marijuana were obtained from the Colorado Department of Revenue and a gravity model was used to generate population-weighted access to retail locations. We tested for spatial autocorrelation using Moran's I and used spatial simultaneous autoregressive lag models to test the association between sociodemographic predictors and spatial access. We found that access to alcohol, marijuana, and tobacco retail stores in Denver was heterogeneous and spatially autocorrelated. Areas of greater access included Downtown Denver and west of I-25. Percent black and percent Hispanic or Latino were negatively associated with access. Conversely, the percent low income was positively associated with access. Education and unemployment were not found to be significant predictors of access in any model. These results demonstrate that black and Hispanic or Latino populations in Denver have decreased access to substances, which is likely a manifestation of decreased access to goods and services more broadly, while low-income populations have greater access and may be at a greater risk for substance use and abuse. Research support provided by the Colorado Department of Public Health and Environment.

Research Associate/ ERHS

O-12. Tertiary lymphoid structures in canine soft tissue sarcomas: characterization and effect on prognosis

Sophia Torres, Kristin Rugh, Laura Ashton, Paula Schaffer, Christine Olver

Tertiary lymphoid structures (TLS) are transient microenvironmental aggregates of immune cells occurring in sites of chronic inflammation, including cancer, external to secondary lymphoid tissue. They are associated with improved prognosis in human sarcomas, which have similar features to canine soft tissue sarcomas (STS). Our objectives were to evaluate the prevalence of TLS within soft-tissue sarcomas, characterize their RNA and protein expression, and gather their effect on prognosis in canines with STS. RNA expression in lymphoid aggregates and adjacent tumor tissue were measured in laser-capture microdissected FFPE tissue and compared to curl-derived RNA from control tissues. Marker expression was quantified using immunohistochemistry and digital image analysis. A retrospective study evaluated prognosis using data derived from the medical records of STS submitted through the CSU Veterinary Diagnostic Laboratory. The prevalence of tumors with at least one suspected TLS was 36 percent. B cells were concentrated in lymphoid aggregates compared with adjacent sarcoma tissue. Plasma cells and high endothelial venules, important markers of TLS, were seen within aggregate-containing tumors but not in control sarcomas Survival times and recurrence did differ between TLS-containing and non-TLS groups. We conclude that the lymphoid aggregates found within canine STS are compatible with TLS. Future work on this project aims to describe the immune contexture within TLS with the hopes of determining whether the immune environment is susceptible to immunotherapies related to checkpoint inhibition. Student supported by NIH training grant T35OD015130.

DVM Student/MIP

O-13. Designing and implementing human factor simulation for assessing optimal PPE behaviors

Quinn Treuting, Shana Gillette, Sangeeta Rao

During livestock disease outbreaks, field staff may interpret the PPE donning/doffing protocol differently. To address this, we utilized human behavior studies, suggesting that visual signs and verbal cues aid in protocol recall. We hypothesized that implementing such cues would improve staff adherence to the PPE protocol during outbreaks. The project aimed to evaluate visual and verbal supportive intervention aids' effectiveness in encouraging appropriate PPE behaviors in a simulated disease outbreak setting. Using a behavioral approach, we tested visual signs and verbal cues in a high-pathogenic avian influenza outbreak simulation on a poultry farm. The aids aimed to promote consistent protocol compliance, even under stress. The study involved 14 government volunteers, split into three groups: control, visual aid, and visual + verbal aid groups. Data collection included post-simulation interviews/surveys (14 completed), task load indices, and randomized video reviews (3 reviewers) to assess the aids' impact. Results demonstrated that both visual and verbal aids improved PPE donning and doffing behaviors, lowering contamination potential. Volunteers using aids experienced reduced mental demand, effort, and frustrations according to the NASA task load index. Post-simulation surveys revealed a preference for visual aids. These findings have practical implications for outbreak simulations' PPE protocols, emphasizing the need for more visual or verbal aids and staff education. Further research should explore physical aids and contamination reduction strategies. Enhancing PPE compliance can better equip staff to manage livestock disease outbreaks effectively.

DVM/MPH Student/ CS

O-14. Characteristics of the canine and feline trauma patients served by emergency and critical care services pre- and post the COVID-19 pandemic in Colorado

Kimona Cameron, Kelly Hall, Tracy Webb, Sangeeta Rao

Dogs and cats across the US are commonly seen by veterinarians in small animal practices for traumatic injuries. These traumatic injuries are one of the leading causes of fatality in these species. Trauma registries are a valuable source of information to characterize the common injuries seen at clinics, and patient clinical course and outcome. The American College of Veterinary Emergency and Critical Care Veterinary Committee on Trauma (VetCOT) has created a multi-institutional database to collect data on traumatic injury cases around the world from veterinary trauma centers to inform clinical practice and improve patient outcome. The purpose of this study is to compare, contrast and characterize the population being served by the Colorado State University Veterinary Trauma Center pre- and post-the 2020 COVID-19 pandemic. The data was retrieved from the VetCOT database and includes the data years January-December 2019-2022. Among the measures being explored are the most prevalent mechanisms of trauma observed throughout the calendar year, injury severity, and patient outcomes (hospitalization, surgical intervention, pharmaceutical treatment, and patient disposition). The analyses on associations between these variables is still being performed. This project was funded in part by a Young Investigator Grant from the Center for Companion Animal Studies.

DVM Student/CS

O-15. Heart of the matter: sex-specific cardiac remodeling in aged rats exposed to early-life chronic stress is predicted by glucocorticoid responsivity

Carley Dearing, Ella Sandford, Nicolette Olmstead, Rachel Morano, Lawson Wulsin, Brent Myers

Cardiovascular disease (CVD) is a leading cause of death worldwide. Additionally, the incidence of CVD varies across the lifespan and between sexes. While the progression of CVD is multifactorial, prolonged stress exposure is a prominent risk factor for adverse cardiac events. The interactions of sex, stress, and aging that impact cardiac susceptibility remain to be determined. To this end, we examined hypertrophy of the aged myocardium in a sex- and stress-specific manner. We hypothesized that sex and stress history would interact to predict cardiac outcomes and that susceptibility or resilience would associate with behavioral, endocrine, and metabolic parameters assessed across the lifespan. In this study, rats were either exposed to chronic variable stress (CVS) in late adolescence (n = 36/sex) or remained as unstressed controls (n = 24/sex). Rats were subsequently challenged with a forced swim test (FST) and glucose tolerance test (GTT) before ageing 15 months and being challenged again. Cardiac tissue was processed for histological analysis and quantification of ventricular morphology. These data indicate early-life chronic stress had sex-specific consequences on cardiac remodeling of the left ventricle with CVS females having greater concentric hypertrophy. Based on inward remodeling, rats were then classified as susceptible or resilient. Multiple behavioral and endocrine factors in young and aged animals significantly interacted with hypertrophy, including coping behaviors and glucocorticoid responses to hyperglycemia. However, correlations with hypertrophy were only present in males. In unstressed young males, baseline corticosterone was positively correlated to left ventricular hypertrophy. Alternatively, CVS males had a positive correlation between visceral adiposity and hypertrophy. Thus, sex-specific cardiac susceptibility or resilience relates to endocrine and metabolic function depending on stress history. This outcome suggests that adverse experiences can have lasting impacts on homeostatic adaptation. Ultimately, these results highlight the importance of sex and prior stress exposure for disparities in cardiovascular risk. Research support provided by NIH grant F30 OD032120 to C. Dearing and NIH grant R01 HL150559 to B. Myers.

DVM/PhD Student/ BMS

O-16. Preferred treatment choices for trauma patients to improve survival outcomes: a pilot study

Ariana Dickson, Kelly Hall, Sangeeta Rao

Trauma is one of the major causes for domestic dog hospitalization and mortality. Treatment choices along with trauma type and severity plays a critical role in recovery and hospital duration of these patients. This pilot study evaluated preferred treatment choices, with a focus on antimicrobials, made by veterinarians based on severity, type of injury, clinical metrics, and their associated survival outcomes in domestic dogs. Data were extracted from the Veterinary Emergency and Critical Care Veterinary Committee on Trauma (VetCOT) database and focused on cases at Colorado State University (CSU) Veterinary Teaching hospital. Treatment data was then extracted from the invoices from the CSU Electronic Medical Record (EMR) and matched with VetCOT Trauma cases. Data analysis was focused on trauma indices, medication, blood values, prognosis, and trauma outcomes. The data was classified into various trauma types and severity and analyzed to assess the distribution and association with certain class of antimicrobials and outcomes. Trauma severity indices used include the Modified Glasgow Coma Scale (MGCS), Animal Trauma Triage (ATT) scores, level of consciousness, evidence of head injury, and blood lactate levels. We analyzed a total of 1,285 canine trauma cases with 50.7% (n= 652) of blunt trauma, followed by 48.3% (n = 621) of penetrating trauma cases and 0.9% (n= 12) both penetrating and blunt trauma that presented to CSU from August 1, 2019 to July 31, 2021. Analysis is still ongoing but the likelihood of use of certain class of drugs with the presence of certain trauma types will be evaluated using a Logistic regression analysis with survival to discharge as the outcome. Research support provided by a Young Investigator award from the Center for Companion Animals.

DVM Student/CS

O-17. Effect of neuroinvasion on strain property maintenance for two α-synuclein prion strains

Sara A. M. Holec, Chase R. Khedmatgozar, Tiffany Pham, and Amanda L. Woerman

Synucleinopathy patients exhibit heterogeneity in clinical presentation, neuropathology, and disease pathogenesis, complicating antemortem diagnoses and therapeutic development. Patients can present with central autonomic dysfunction or peripheral dysfunction that converts to a central disease. While the exposure route influences classic prion pathogenesis, it is unknown if or how peripheral exposure alters the disease pathogenesis of α-synuclein prion strains. We hypothesized that, unlike some PrP strains, two α-synuclein strains will maintain their properties during neuroinvasion. To test this, we inoculated either 20 µL of mouse-passaged MSA (multiple system atrophy) brain homogenate or recombinant A53T preformed fibrils (PFFs) into TgM83+/- mice and compared the onset of neurological disease between sciatic nerve (sc.n.) and intracranial (i.c.) injections. Brains from terminal animals were collected half frozen/half fixed for subsequent analyses. Spinal columns of sc.n.-injected mice were also collected and fixed for immunohistochemistry. Homogenates were tested for infectivity in a panel of α-syn140-YFP biosensor cell lines. As anticipated, A53T PFFs transmitted disease to TgM83+/- mice with a shorter incubation period than MSA prions, regardless of injection route. Notably, the incubation period was extended by ~50% following sc.n. injection, regardless of strain. A-synuclein prions isolated from brain tissue were similarly infective in the α-syn140*A53T-YFP biosensor cells, regardless of strain, suggesting α-synuclein prion content is consistent in terminal mice. However, strain-specific differences were observed across a panel of cell lines, indicating distinct biological activities. This is consistent with the observation that the MSA strain was more sensitive to proteinase K digestion, but more resistant to guanidine denaturation, compared to the passaged A53T PFFs. Despite these differences, mice developed similar neuropathology throughout the hindbrain, regardless of exposure route or strain. Our findings suggest that the location of disease origin does not alter MSA prion pathogenesis, which has important implications for treating patients with peripheral synucleinopathy that converts into MSA. This project was funded by NIH R01NS121294-02, R01NS121294-02S1.

Post-doctoral Fellow/ MIP

O-18. Evaluating dairy farming interests of K-12 and college students utilizing virtual reality as an educational tool

Elizabeth Kim, Pedro Boscan, Isaac Fraire, Morgan Jones, Cyane Tornatzky, Aracely Diaz, Marie Vans, Wenjing Jiang, Sangeeta Rao

Agriculture-focused education in K-12 curriculum is in dearth, directly impacting a student's interest in agricultural pursuits. This impact can especially be seen within livestock agriculture, contributing to the decreased workforce as younger individuals increasingly gravitate towards alternative urban activities and employment opportunities. Therefore, it is an emerging issue in agriculture that needs special attention. There is a need for targeted interventions to instill a renewed interest in livestock agriculture among the younger generation. To address this challenge, our project focused on harnessing the potential of virtual reality (VR) technology as an innovative teaching tool within the K-12 curriculum. As the education system explores the vast possibilities offered by the virtual realm, this study sought to evaluate the feasibility, applicability, and likeability of an interactive VR farming game for K-12 and college students. The VR tool developed for this study employs a concept process algorithm where participants are placed in a locker room within a dairy farm with options to choose from a variety of clothing and protective equipment to wear. Participants need to choose the appropriate items in their correct sequence to progress further into the farm. To assess the tool's effectiveness, a questionnaire was administered to the participants after completion of the game. Data from students helped to assess the likeability and whether VR could be an effective educational training tool for animal agriculture. Preliminary data from 18 participants reported an enjoyable VR experience (average ± SD: 8.9 ± 1.30), and 89% answered "Yes" when asked whether they learned from the VR game. These findings suggest favorable effectiveness of VR technology as an educational tool and to evaluate dairy farming interests among this younger population. Further data is necessary to enhance the VR training platform in expanding and encompassing a broader spectrum of livestock practices within the farm environment. Research support provided by High Plains Intermountain Center for Agricultural Health and Safety (HICAHS) Emerging Issues Program.

DVM Student/CS

O-19. A tail of two calves: an epigenetic timeline of BVDV infections

Jessica N. Kincade, Jeanette V. Bishop, Hanah M. Georges, Carolina L. Gonzalez-Berrios, Terry E. Engle, Hana Van Campen, and Thomas R. Hansen

Bovine viral diarrhea virus (BVDV) costs the cattle industry billions annually. BVDV crosses the placenta and induces early embryonic death, abortion, and the generation of weak, non-viable calves. Fetuses infected before 125 days of gestation cannot mount a complete adaptive immune response and develop an immunotolerance to the virus. Postnatally, persistently infected (PI) calves often have congenital deformities and chronic immunosuppression. When fetal infection occurs post 150 days of gestation, the adaptive immune response is more robust and includes the production of antigen specific antibodies capable of clearing the virus. Fetal transient infections (TI) produce underweight calves with impaired growth rates. Because PI fetuses demonstrate epigenomic alterations consistent with known postnatal pathologies, it was hypothesized that BVDV induces epigenetic changes that persist throughout postnatal life. To test this hypothesis, BVDV TI cattle and control cattle were generated while PI cattle were identified on a local, cooperating ranch. Peripheral blood mononuclear cells (PBMCs) from 4 month old TI, PI, and Control cattle were analyzed for differential methylation. Of the 2,640 differentially methylated sites (DMSs) previously identified in fetal spleen at 245 days of gestation and the 4,921 DMSs identified in PBMCs at 4 months of age, 670 genes in common between datasets were found to contain at least 1 DMS. Of the 2,326 DMSs identified at birth and the 4,015 DMSs identified at 4 months of age in TI calves, 616 DMSs were found on genes common to both datasets. The existence of DMSs prior to influence of the external environment indicates that fetal BVDV infection leads to the alteration of DNA methylation. Additional DMSs are evident at 4 months of age, suggesting the potential to influence both TI and PI calves into the postnatal period through fetal programming. This research was supported by USDA NIFA Grants: 2019-67015-29866, 2021-38420-34040, and 2023-67011-40513.

Graduate Student/ BMS

O-20. Adaptation of non-lymphotropic emergent Nordic CWD to a lymphotropic strain

Diana C. Lowe, Julianna Sun, Sehun Kim, Jenna Crowell, Emma Raisley, Bailey Webster, Jifeng Bian, Erin Flaherty, Chloe Smith, Hannah Bodrogi, Maria Nöremark, Dolores Gavier-Widen, Sylvie Benestad and Glenn Telling

In previous studies of emergent chronic wasting disease (CWD) in Norway, Sweden and Finland, we found CWD from Nordic cervids to be a biologically unstable, unique, and highly diverse form of CWD distinct from North American (NA) CWD. Unstable strains with unknown host ranges pose a threat to sympatric wildlife and humans, making the characterization of emergent strains a high priority. Established NA CWD is characterized by high levels of prion infection in the lymphoreticular system, a feature that contributes to the highly contagious nature of the disease. Although Nordic CWD cases seem to be non-lymphotropic, the potential of Nordic CWD to adapt and eventually acquire tropism for lymphoid tissues is unknown. To determine if Nordic CWD prions can acquire de novo lymphotropism, we performed transmission studies of CNS tissue of the four reported Swedish moose cases in gene targeted (Gt) mouse models, which express cervid PrP at physiological levels. We collected spleens from all mice diagnosed with prion disease. To assess presence of prion infectivity, we purified PrPSc from spleen homogenates by ultracentrifugation and performed SDS-PAGE and Western blot and RT-QuIC assays. We found the first Swedish moose isolate contains a strain capable of replication in the spleen when passaged in Gt mice expressing cervid PrP with glutamic acid at residue 226. Future studies will explore the interspecies transmission potential of this new lymphotropic CWD strain. Gt mice are a valuable tool to predict the evolution and adaptation of prion strains in the wild and their zoonotic potential. Research support provided by NIH. Student support provided by NIH Training Grant T32GM132057.

Graduate Student/MIP

O-21. Denver's tree canopy coverage: perspectives from the Globeville, Elyria, Swansea community health study

Elizabeth Lunsford, Sheryl Magzamen

Purpose: The concept of greenspace access as a therapeutic medium for health has been well established. However, greenspace access within formerly redlined communities may not result in greater health benefits due to current social stressors present in marginalized communities. The objective of this study is to determine if greenspace measured by tree canopy coverage in Denver is associated with historical redlining, adjusting for present-day neighborhood sociodemographics. We hypothesized that there is an inverse relationship between tree canopy coverage and selected demographic characteristics and redlining status. Methods: We used publicly available data to statistically model the relationship between tree canopy coverage and historical redlining, ethnicity, and poverty in Denver using multiple weighted least square regression. Results: Regression results indicate that there is significantly lower tree coverage among neighborhoods with greater concentrations of Hispanic/Latino populations and those living below 100% poverty. Tree canopy coverage in HOLC Grade D block groups (i.e., lowest rated neighborhoods) is 14.3% (95% CI: -16.1, -12.6) less than HOLC Grade A (i.e., highest rated neighborhoods) while controlling for percent poverty. Tree canopy coverage in HOLC Grade D block groups is 12.3% (95% CI: -14.0, -10.6) less than HOLC Grade A while controlling for percent Hispanic/Latino. The percent Hispanic/Latino and percent below 100% poverty were significantly associated with decreased tree coverage (19% (95% CI: -20.8, -17.1), and 72.3% (95% CI: -84.9, -59.8) respectively). Conclusion: Denver's historically redlined census tracts have a higher percentage of Hispanic/Latino residents and residents living below 100% poverty while also experiencing lower tree canopy coverage. Efforts to move Denver towards environmental equity can start by focusing on improving tree canopy as a health and environmental measure. Funding for the study was through the Globeville, Elyria, and Swansea Community Health Study.

Graduate Student/ ERHS

O-22. West Nile Virus Mosquito Vector Changes and Land Use Changes in Fort Collins, Colorado, 2006-2021

Courtney Maichak, Elizabeth Lunsford, Michael Young, Broox Boze, Gregory D. Ebel, SueVandeWoude, Sheryl Magzamen

Landscape epidemiology analyzes temporal factors of the three main points of the disease triad (host, vector, and pathogen), and assesses how these factors interact spatially within the environment to facilitate pathogen transmission. Application of this analytic framework can be beneficial for West Nile Virus (WNV), which was initially introduced to the United States in 1999, spreading westward into Colorado by 2003 and since has remained endemic. The aim of our study was to see how land use change in Fort Collins, Colorado, a city undergoing rapid development, impacted two WNV vectors, *Culex pipiens* and *Culex tarsalis*, over a fifteen-year study period. The two species have shown different habitat preferences between urban and rural land cover, and different competence as vectors for WNV. Spatial regression using linear mixed models to compare the collection abundance change over the study period by collection sites (n=43) and city zones (n=4). Land cover evaluation was completed using Multi-Resolution Land Characteristics Consortium National Land Cover Database using six years of 30 meter resolution rasters, which were then classified as developed v. undeveloped. Average daily flight of 1km was added around each collection site to evaluate land use within the area. A total of 8,429 observations were collected over the fifteen year period. Preliminary regression analysis found that count of *Cx. pipiens* increased an average of 47.57 (95%CI: 43.71 - 51.43) specimens if sites were classified as developed v. undeveloped whereas *Cx. tarsalis* decreased by an average 245.52 (95%CI: -246.02 - -245.02) specimens per site classified as developed v. undeveloped. This switch of vector abundance within the study could alter the transmission of WNV and require a change to the control methods used in the area. Controlling these species efficiently can provide protection to at risk populations of both humans and equids from contracting WNV. Research support provided by the Colorado State University One Health Institute.

Graduate Student/ERHS

O-23. Pathogenesis and transmission of severe fever with thrombocytopenia syndrome virus in experimentally infected animals

Jeffrey M. Marano, Airn E. Hartwig, Stephanie M. Porter, Nicole M. Nemeth, Marissa Quilici, Angela M. Bosco-Lauth

Severe fever with thrombocytopenia syndrome virus (SFTSV; order Bunyavirales, family Phenuiviridae, genus Banyangvirus) is a newly recognized arbovirus of significant human health concern. Since its discovery in 2009, there have been over 15,000 cases across Eastern Asia, with case fatalities primarily in people over the age of 50. With this evolving threat, significant gaps exist regarding the pathogenesis, serological surveillance, and transmission dynamics of SFTSV. To address these gaps, we explored the pathogenesis and serology of SFTSV in juvenile cats, as they are natural hosts for the virus and present with similar symptoms to humans. Our data demonstrate that all the directly infected cats demonstrated liver pathologies and seroconverted, and 4/8 of directly infected cats became viremic, panleukopenic, and febrile, but ultimately recovered. Serum from these cats was then used to establish serological diagnostic criteria similar to what has been previously reported for dengue virus. We demonstrated a >4-fold difference in PRNT90 values for SFTSV and Heartland virus, an endemic bandavirus within the United States. These results provide a framework for wildlife surveillance of SFTSV to monitor its potential emergence in the United States. During our initial pathogenesis studies, we observed that one of our uninfected "contact" cats contracted SFTSV and ultimately succumbed to the disease. This is notable, as this infection occurred without a vector. These findings align with previous reports of nosocomial infections in human and animal patients and the occupational infections of veterinarians and health care staff after encountering SFTSV-positive patients. To identify these non-canonical routes of infection, we performed intramuscular, ocular, intranasal, and oral infections using several animal models. We demonstrated that non-canonical routes of inoculation can result in infection and severe disease. These studies provide the necessary foundations for surveillance and preparedness for the United States to respond to the possible emergence of SFTSV. Research support provided by NIH R21 AI146500.

Post-doctoral Fellow/ BMS

O-24. MR-1 antigenicity is dependent on riboflavin biosynthesis in *Mycobacterium tuberculosis*

Nurudeen Oketade, Melissa D. Chengalroyen, Aneta Worley, Megan Lucas, Luisa Nieto Ramirez, Mabule L. Raphela, Gwendolyn M. Swarbrick, Digby F. Warner, Deborah A Lewinsohn, Carolina Mehaffy, Valerie Mizrahi, David M. Lewinsohn, Karen Dobos

Mycobacterium tuberculosis (*M.tb*), the causative organism of tuberculosis, infects 7 million people and causes 1.7 million deaths yearly. Currently, there is only one approved vaccine against *M.tb*, bacillus Calmette-Guerin (BCG), which has limited efficacy and provides limited protection. This has made the development of an effective vaccine against *M.tb* a priority. The discovery of MHC-1-related protein (MR-1) restricted T cells, a subset of T cells that recognize metabolic antigens and their protective trait during infectious disease and cancer, has made them a prime target for vaccine development. Most of the metabolic antigens recognized by MR-1 T cells identified to date come from the intermediates of the riboflavin biosynthetic pathway (RBP), a pathway that is absent in humans. Since *M.tb* has a functional RBP, we hypothesize that *M.tb* will produce metabolites recognized by MR-1 T cells. Using loss of function and complementation studies combined with the EliSPOT immunoassays, we show that MR-1 antigenicity depends on the RBP in *M.tb*. The knockout of the *ribA2* gene, which encodes for the first enzyme in this pathway, led to the complete loss of MR-1 antigenicity. However, loss of antigenicity was not observed when we knocked out the ribH gene, which encodes for the subsequent enzyme in this pathway. This result indicated that the enzymatic product of the RibA2 enzyme, 5-amino-6-(D-ribityl amino) uracil (5-A-RU), played a crucial role in MR-1 antigenicity in *M.tb*. In support of this finding, we also showed that the complementation of *M.tb ΔribA2* with endogenous 5-A-RU led to the recovery of MR-1 antigenicity. Interestingly, a previous study has shown that 5-A-RU lacks MR-1 antigenic properties. We, therefore, hypothesize that 5-A-RU is being trafficked to other metabolic pathways to generate MR-1 antigens. Future studies will look to identify the downstream products derived from 5-A-RU and their contribution to MR-1 antigenicity in *M.tb*. Research support provided by the grant, "T cell recognition of the MR1 presented microbial metabolome", U Chicago (Adams, NIAID PI), award # AWD100279 to KMD.

Graduate Student/MIP

O-25. Metabolic makeover: Investigating the role of metabolic remodeling in the mosquito vector during arbovirus infections

Oshani C. Ratnayake, Paul S. Soma, Nunya Chotiwan, Samantha Pinto, Irma Sanchez Vargas, Barbara Graham, Elizabeth McGraw, Rushika Perera

Dengue, Zika (flaviviruses) and chikungunya (alphaviruses) are among the most aggressive arboviruses transmitted by the bite of an infected Aedes aegypti (Ae. aegypti) mosquito. These viruses cause a spectrum of symptoms from self-limiting febrile diseases to fatal hemorrhagic fever, neurological disorders or chronic arthritis. In the absence of successful antivirals or effective vaccines, these viruses remain a significant health burden affecting nearly four million people, especially in developing countries. Using systems biology, we have shown that arboviruses, dengue, Zika and chikungunya, significantly alter the metabolome of Ae. aegypti during infection. The results show key changes in bioactive fatty acids, sphingolipids and glycerolipids as well as changes in structural glycerophospholipids and sterols. Interestingly, some metabolic pathways are altered similarly by all arboviruses tested, while other pathways seem specific to one virus indicating common and diverse virus-vector interactions. A key pathway of interest is Glycerolipid (GL) metabolism. Glycerolipids consist of triacylglycerols (TAG), the major lipid component of the mosquito lipophorin (responsible for lipid transport in the hemolymph) and its metabolites, monoacylglycerols (MAG) and diacylglycerols (DAG)). TAG plays a significant role in energy metabolism, can be stored in the fat body as an energy reserve or be transported to the ovaries for vitellogenesis. In our studies, we detected increased abundance of TAGs during infection with all arboviruses, and this increase remained constant during a time course of infection. Higher levels of TAGs are achieved by conversion of MAGs and DAGs via diacylglycerol o-acyltransferase (DGAT) activity as a response to infection. We are exploring the interaction between glycerolipid metabolism and vector immunity and its impact on infection success. We are using loss of function studies (double-stranded RNA, CRISPR-CAS and inhibitors) to probe this metabolic pathway. Our goal is to identify metabolic choke points in the mosquito vector that can be effective in vector control. Research support provided by NIH/NIAID 1RO1A151166-01.

Graduate Student/ MIP

O-26. Susceptibility of ectotherms and house sparrows to Japanese encephalitis virus (JEV) genotypes I-IV

Ashley Walker, Airn E. Hartwig, Angela M. Bosco-Lauth, Richard A. Bowen

Japanese encephalitis virus (JEV) is a vector-borne flavivirus that is known to be maintained in an enzootic lifecycle between mosquitoes, pigs and wading birds. An estimated 68,000 human cases are reported annually, and symptoms in humans can range from a mild fever to severe neurological complications. Arboviral diseases are spreading to new areas at alarming rates secondary to increases in global trade and travel, migration of animal reservoirs and climate change. Although JEV is currently only endemic in Asia, concerns for the spread of JEV into new areas are rising as indicated by a recent outbreak in Australia. This outbreak is causing significant public health impacts by inflicting illness in humans and creating notable economic losses to the pig industry. Given that other closely related arboviruses such as West Nile virus have spread to the U.S. over the past several decades, JEV holds high potential to become established in the U.S. However, little is known about what animal reservoirs, particularly wildlife inhabiting mosquito-dense locales, could contribute to JEV ecology in the U.S. Therefore, we assessed the susceptibility of house sparrows and several North American ectotherm species to the four main genotypes of JEV circulating in endemic areas. Here we report that ball pythons, garter snakes, frogs and house sparrows are susceptible to JEV genotypes I and III, but not to JEV genotypes II and IV. Frogs exhibited susceptibility to JEV genotype I while toads were not susceptible to any of the four genotypes of JEV. Our results expand upon the knowledge base of susceptible species and provide evidence that domestic wildlife species could play a role in the introduction or maintenance of JEV within the U.S. Research support provided by the USDA.

Graduate Student/BMS

O-27. Evaluation of viscoelastic testing and coagulation/hemostasis data in a canine hemorrhagic shock/resuscitation model

Taylor N. Baird, Kristin Zersen, Julien Guillaumin

To assess changes in viscoelastic testing and traditional hemostasis tests in a canine model of pressure-target hemorrhagic shock, eight healthy dogs were anesthetized and instrumented. After a 10-minute period of blood pressure stabilization (T1), dogs were hemorrhaged to a fixed mean arterial blood pressure of 40±5 mmHg for 10 minutes (T2). Dogs were then resuscitated with 100% of shed blood (T3). Complete blood count, prothrombin time (PT), partial thromboplastin time PTT), fibrinogen, antithrombin and D-Dimers level, and the VCM-Vet™ parameters Clot Time (CT), Clot Formation Time (CFT), Angle, Maximum Clot Formation (MCF), Amplitude at 10 and 20 minutes (A10 and A20, respectively) and clot lysis index at 30 and 45 minutes (LI30 and LI45, respectively) were performed at T1, T2 and T3. Friedman tests were performed. CT (min) was 3.7 [2.1–7.3], 4.2 [2.2–6.0] and 2.0 [1.3–6.4] while CFT (min) was 4.4 [2.4–10.3], 3.0 [2.0–9.2] and 3.2 [1.3–9.4] at T1, T2, and T3, respectively (p=1.000 for both). Median angle (degree) was 46.5 [24.0–61.0], 53.0 [28.0–62.0], and 54.5 [35.0-71.0] at T1, T2, T3, respectively (p<0.001). Median A10 and A20 (unit) were 16.5 [10.0–26.0], 20.5 [11.0–29.0], 19.0 [10.0–32.0] (p<0.001) and 24.0 [15.0–35.0], 26.5 [16.0–36.0] and 25.5 [14.0–37.0] (p<0.001), respectively, at T1, T2, T3 respectively. MCF was 30.0 [20.0–44.0], 33.0 [23.0–44.0], 35.0 [19.0–66.0] at T1, T2, T3, respectively (p=1.000). Median Li30 (%) and Li45 (%) were 100 [66–100], 100 [98–100], 100 [39–100] (p <0.001) and 100 [73–100], 100 [88–100], 100 [45–100] at T1, T2, T3, respectively. Significant prolongation of PTT occurred (p <0.001). No significant changes in PT, fibrinogen, antithrombin, or D-Dimers were seen. We demonstrated coagulopathy consistent with hypercoagulability and an increased PTT in our hemorrhagic shock model

Resident/ CS

O-28.Arterial blood gas parameters in healthy, acclimatized non-brachycephalic and brachycephalic dogs at a 1,535 altitude

Charles T. Talbot, Kristin M. Zersen, Billy Poon, Kelly S. Santangelo, A Russell Moore, Amanda A. Cavanag

An arterial blood gas (aBG) is a point-of-care diagnostic test used to evaluate oxygenation, ventilation, and acid base status. Studies have identified an inverse relationship between altitude and partial pressure of arterial oxygen (PaO2), arterial oxygen saturation (SaO2), and partial pressure of carbon dioxide (PaCO2). The primary purpose of this study was to define reference intervals for aBG parameters in healthy, acclimatized, non-brachycephalic, non-sedated dogs living at 1,535 meters above sea level. We hypothesized these dogs would have a lower PaO2, SaO2, PaCO2, and HCO3, with higher pH, hemoglobin (ctHb), and systolic blood pressure (sBP), compared to dogs at sea level. The secondary purpose was to evaluate and compare aBG values for healthy, acclimatized, non-sedated, brachycephalic dogs to non-brachycephalic dogs. We hypothesized that brachycephalic dogs at altitude would have lower PaO2, SaO2, PaCO2, and bicarbonate (HCO3), with higher pH, ctHb, and sBP, compared to non-brachycephalic dogs. 120 healthy, adult dolichocephalic and mesocephalic dogs, and 20 healthy, adult brachycephalic dogs, were enrolled. The mean and reference interval values for pH (7.4424; 7.3751-7.5148), PaO2 (78.77; 59.20-99.82mmHg), PaCO2 (27.99; 21.52-34.39mmHg), SaO2 (98.39; 84.54-101.40%), HCO3 (18.88; 14.92-22.10mmol/L), ctHb (17.46; 13.42-21.10g/dL) and sBP (132.6; 94.1-180.0mmHg) were established for dolichocephalic and mesocephalic dogs at altitude. Brachycephalic dogs had significantly lower PaO2 and SaO2 and significantly higher ctHb compared to non-brachycephalic dogs acclimatized to a similar altitude. aBG reference intervals for healthy dolichocephalic and mesocephalic dogs acclimatized to an altitude of 1535m were established. Healthy dogs at this altitude have a lower mean PaO2 and PaCO2, and higher pH, ctHb, and HCO3 compared to dogs at sea level. Brachycephalic dogs at altitude had significantly different PaO2, SaO2 and ctHb values compared to non-brachycephalic dogs; separate reference intervals should be established for these brachycephalic dogs. This study was supported by a College Research Council grant.

Resident/ CS

O-29. Prevalence and association of pancreatitis in dogs with hypercalcemia

Katherine A Neal, Sangeeta Rao, Angela Marolf

A possible association between pancreatitis and hypercalcemia has been widely debated in human medicine. Objectives of this single-center, retrospective, observational study were threefold: to estimate the prevalence of pancreatitis in dogs with hypercalcemia, to determine if a specific etiology/etiologies have a higher prevalence of pancreatitis, and to determine if higher levels of serum calcium was more likely to be associated with pancreatitis. Medical records (January 2005 – December 2021) were searched to identify dogs with hypercalcemia that met the inclusion criteria of 1) abdominal ultrasound and 2) ionized calcium and serum calcium levels obtained within 1 week of imaging (n = 180). The included dogs were categorized by the etiology causing hypercalcemia (primary hyperparathyroidism, malignancy, renal hyperparathyroidism, hypoadrenocorticism, and granulomatous disease). Ultrasound images were reviewed for evidence of pancreatitis by a board-certified veterinary radiologist and a veterinary diagnostic imaging resident. The overall prevalence of pancreatitis in dogs with hypercalcemia was 18/180 dogs (10%). Prevalence of pancreatitis in each category is as follows: primary hyperparathyroidism, 10/63 (15.87%), hypercalcemia of malignancy, 6/106 (5.66%), renal hyperparathyroidism, 1/6 (16.67%), hypoadrenocorticism, 1/3 (33.33%), and granulomatous disease, 0/2 (0%). Based on logistic regression analysis, dogs with primary hyperparathyroidism were more likely to have pancreatitis than dogs with hypercalcemia of malignancy ($p < 0.033$). Hypercalcemic dogs with pancreatitis had significantly lower ionized calcium ($p < 0.0042$) and serum calcium ($p < 0.0056$) than hypercalcemic dogs without pancreatitis. Pancreatitis may be associated with lower calcium levels in hypercalcemic dogs; however, further studies are needed to elucidate these findings.

Resident/ ERHS

O-30. The use of doxorubicin and propranolol for canine splenic hemangiosarcoma: a retrospective study of 31 dogs

Trish E. Paulos, Sarah M. Tan, Douglas H. Thamm

Canine splenic hemangiosarcoma (HSA) typically has a poor prognosis despite multimodal treatment. Recent research suggests that the beta-adrenergic receptor antagonist propranolol may decrease chemotherapy resistance in canine sarcoma cell lines in vitro and may have immunomodulatory and antiangiogenic activity. The objective of this retrospective study was to evaluate the safety and oncologic outcome in dogs with splenic HSA treated with a combination of propranolol and doxorubicin. Medical records of 31 dogs were reviewed for this study. Dogs were included if they had a confirmed diagnosis of grade I or II splenic HSA and were treated with splenectomy followed by doxorubicin and propranolol. Propranolol was administered at a target dose of 1 mg/kg TID. Adverse events (AEs) were graded via VCOG criteria. Progression-free interval (PFI) and overall survival time (OST) were estimated via the Kaplan-Meier method. Median PFI was 160 days and median OST was 182 days. Dogs with stage I HSA had prolonged median PFI and OST of 421 days while those with stage II disease had a median PFI and OST and of 153 days. AEs documented in this study were mostly secondary to doxorubicin administration though three dogs experienced AE attributed to propranolol leading to discontinuation of the medication. Although reported outcomes in this study group are comparable to those reported with doxorubicin-based chemotherapy alone, these data suggest that combined doxorubicin and propranolol appears safe and well tolerated.

Resident/CS

O-31. Ancillary treatments with thrombolytics or medications aimed at improving functional recovery or both in acute feline aortic thromboembolism

Christopher C. Ray, Jake Wolf, Julien Guillaumin

This is a retrospective, bicentric study investigating cats with acute cardiogenic feline aortic thromboembolism (FATE) receiving tissue plasminogen-activator (tPA) continuous rate infusion (CRI) or medications aimed at improving functional recovery, specifically pentoxifylline and/or cyproheptadine, or both. Inclusion criteria were any cat presenting for cardiogenic FATE that received any of the following medications: tPA-CRI, pentoxifylline or cyproheptadine. Survival to discharge and functional recovery in our study population was compared to the 1-hr tPA or placebo control group derived from a published prospective multicenter study using a Fisher exact test. Seven cats (57% male cats) with cardiogenic FATE were enrolled. Median age was 6-years and median weight was 5.4kg. Most cats suffered from bilateral FATE (83%) while 1 cat (17%) had a left hindlimb FATE. At admission, median rectal temperature was 98.5°F, median lactate of affected limb was 12.1 mmol/L; median creatinine was 1.3 mg/dL: and median potassium was 3.7 mmol/L. Five cats (71%) received tPA-CRI, using a dose of 0.1mg/kg/hr for a total of 1 mg/kg. Four cats (57%) received pentoxifylline (60-100mg q12hr) and four cats received cyproheptadine (2mg q12hr). Six cats received combination therapies, with three receiving all three therapies. Reperfusion injury and acute kidney injury were documented in 50% and 29% of the cats, respectively. All study cats (100%) had an improvement in locomotion, compared to 60% in the historical 1-hr TPA group (p=0.068) and 40% in the historical placebo group (p=0.08). The improvement was classified as mild (43%), moderate (14%), or marked (43%). Four cats survived to discharge (57%), compared to 45% in the 1-hr TPA group (p=0.678) and 30% in the placebo group (p=0.365). In conclusion, the therapies described resulted in a higher proportion of cats regaining motor function compared to a historical placebo group. The exact impact of each therapy is unknown.

Resident/ CS

O-32. Evaluating the efficacy of shelf stable blood products for resuscitation in a canine hemorrhagic shock model; an endothelial glycocalyx evaluation

Mark Ryan, Rebekah Ford, Kelly Hall, Julien Guillaumin, Emily Venn, Tom Edwards, Guillaume Hoareau

Hemorrhagic shock causes significant morbidity and mortality in canine trauma patients. Current veterinary resuscitation methods require temperature-controlled blood products with limited lifespan restricting their use in austere environments. Utilizing the GlycoCheck®, this study evaluated whether resuscitation strategies leveraging the stability of freeze-dried plasma (FDP), hemoglobin-based oxygen carrier (HBOC), and lyophilized platelets (LP) provide an alternative to conventional hemorrhage resuscitation strategies. The GlycoCheck® measures the perfused boundary region (PBR) of the endothelial glycocalyx in sublingual capillaries which increases with microcirculatory damage. It's hypothesized that resuscitation with shelf stable products will not significantly alter PBR when compared to conventional blood products. Utilizing a cross over design, 7 purpose bred dogs were anesthetized, instrumented, and subjected to controlled hemorrhage of 40% of their blood volume to achieve a mean arterial blood pressure of 35-50 mmHg. Resuscitation was performed with either Lactated Ringers/Hetastarch (LRS/HES) Solution, CWB, Fresh Frozen Plasma/Packed Red Blood Cells(FFP/pRBC), FDP/HBOC, or FDP/HBOC/LP. The endothelial glycocalyx, as a surrogate for endothelial health, was evaluated by assessing sublingual microcirculation's PBR with a GlycoCheck® prior to hemorrhage (TO), after hemorrhage (T75), after 30 minutes in hemorrhagic shock (T105), after completion of resuscitation protocol (T150) and at conclusion of the procedure (T180). No statistically significant difference was appreciated between PBR of CWB, FFP/pRBC, FDP/HBOC and FDP/HBOC/LP at any time point. Statistical significance between the PBR of CWB and FFP/pRBC was appreciated when compared to LRS/HES at T180. Statistical significance was set at $P<0.05$. In this canine hemorrhagic shock model, no statistical difference in PBR after resuscitation with shelf-stable products versus CWB was appreciated, inferring that they are an acceptable alternative to preserving the endothelial glycocalyx in hemorrhagic shock. The biological relevance of these findings should be determined in an uncontrolled hemorrhage model, however resuscitation with shelf-stable products appears to be satisfactory. Research supported by Award: W81XWH21C0002;VRO: approved (US SOCOM).

Resident/CS

O-33. First Day Readiness-Basic Radiographic Clinical Interpretation-4th year Veterinary Students

Megan Stadler, Andrew West, Linda Dillenbeck, Tiffany Martin, Kayla Woodring, Elissa Randall, Lynn Griffin

First day readiness for veterinarians should include basic abilities to interpret common radiographic studies. Objective evaluation of teaching methodology for radiographic interpretation is currently unavailable. We designed a prospective, randomized controlled study utilizing the CSU class of 2022 (n=147). Clinical rotations were randomly assigned different groups for pretesting: Group A (control, historical non-case-based pretest), Group B (case-based, non-immediate feedback pretest), and Group C students (same case-based questions as group B with immediate feedback and additional, in-depth information provided). All students then participated in the standard clinical rotation. The posttest at the end of the rotation was identical for all groups. The primary objective was to compare the students' posttest improvement performance between groups. The secondary objective was to compare class ranking in the pre-clinical didactic course to the students' improvement scores. We hypothesized that there would be no difference between the different testing methods. There were just under 50 students per group. Comparison of the case-based pretesting groups (B and C) had statistically significant difference, indicating greater improvement scores from the pretest to the post-test ($p=0.0008319$ for B to A and $p=0.0000017$ for C to A). In comparison to group A, a student from group B would score almost 3.68 points (out of 40) higher on the posttest while a student group C would score about 4.85 points higher. There was no difference between Group B and C. For our secondary objectives, Group 1, Group 2 and Group 3 were defined as students who scored a ≥90% in the didactic class, scored < 90% in the class and students who didn't take the class. There was no statistical significance between student status and improvement scores amongst the groups. Case-based pre-tests improved students post-test with no significance between the case-based groups.

Resident/ CS

O-34. Anesthesia of Madagascar Hissing Cockroaches (*Gromphadorhina portentosa*) with Isoflurane, and a Novel Technique for Heart Rate Monitoring

Summer Barnes, Miranda J. Sadar, Jennifer Hausmann, and Khursheed Mama

Information on effectively anesthetizing Madagascar hissing cockroaches (*Gromphadorhina portentosa*) is limited. This study evaluated isoflurane using both physical (e.g., response to noxious stimulus) and physiological (e.g., heart rate [HR]) parameters. Awake HR was recorded in seventeen adult cockroaches (six females and 11 males) using a Doppler. Cockroaches were anesthetized in groups of two or three using 2 mL of isoflurane on a cotton ball, in a 1L chamber. Induction time was recorded when there was no response to external movement of the chamber. Cockroaches were removed from the chamber and exposed to noxious (pinprick) and non-noxious stimuli (Doppler crystal). Heart rates were assessed over 10 seconds every 5 minutes until cockroaches were able to right themselves (recovery time). Normally distributed data were assessed using an unpaired t-test. A non-parametric test was used for not normally distributed data; $p \leq 0.05$. The mean (± standard deviation) induction time was 14.7 ± 4.2 minutes and not significantly affected by sex, length, or weight. Mean recovery time was 21 ± 5.1 minutes. Males ($p=0.007$) and cockroaches > 6g ($p=0.03$) recovered faster. Heart rate was 69 ± 12 bpm and 63 ± 9.6 bpm in awake and anesthetized cockroaches, respectively ($p=0.05$) with no impact of sex, length, or weight. Response to stimulus was observed in seven individuals; three responded to only the non-noxious stimulus, one to only noxious stimulus and three to both. Madagascar hissing cockroaches were successfully anesthetized with isoflurane which should be considered for short term procedures.

DVM Student/CS

O-35. Preliminary data on evaluation of new immunotherapy for cats with FIP being treated with EEID-2801 antiviral

Petra Černá, Steven Dow, McKenna Willis, Kaci M Shaw, Jennifer Hawley and Michael R Lappin

Feline Infectious Peritonitis (FIP) has been a devastating disease among cats for over half a century. Recent studies reported the use of antiviral drugs such as GS-441524 and EEID-2801 as successful treatment of FIP, but only remdesivir is currently legally available in the USA. It has been suggested that antiviral drugs can act synergistically with immunomodulatory treatments to improve patient outcome and survival in different viral diseases. The purpose of this study was to determine the efficacy of the oral new liposome-toll-like receptor agonist (TLR3 and TLR9) complex (LTC) as an adjunct treatment of cats being treated with EEID-2801. A total of 18 cats diagnosed with feline infectious peritonitis were enrolled in the study, 5 of these cats were euthanized or died within the first 48 hours of enrollment and the other 13 cats have completed a minimum of 4 weeks of therapy with repeated bloodwork (8 cats in LTC group and 5 cats in placebo group). Out of the 13 cats, 8 were effusive (7 peritoneal and 1 pleural effusion), 1 cat had dry abdominal form and 4 cats were diagnosed with ocular FIP. At diagnosis, the median serum total protein was 8.1g/dL (IQR 7.4-9.7), median albumin 2.3g/dL (IQR 2.2-2.5), median globulins 5.3g/dL (IQR 4.9-7.2) and median albumin:globulin (A:G) ratio was 0.4 (IQR 0.3-05). At the 4 weeks recheck, the median serum total protein was 7.5g/dL (IQR 6.9-8.0), median albumin 3.3g/dL (IQR 3.0-3.4), median globulins 4.4/dL (IQR 3.8-4.6) and median A:G ratio was 0.7 (IQR 0.7-0.9). All cats were clinically doing well. The only reported side effect was hypersalivation in one cat treated with EEID-2801 and placebo, all the other cats tolerated the therapy well. These preliminary data suggest that these medications are useful in successful treatment of FIP without cats without severe side effects. Funding for this study is provided by Every Cat Health Foundation and the CSU Center for Companion Animal Studies.

Graduate Student/ CS

O-36. Improving health care literacy among patients with multiple sclerosis through a flipped classroom model

Jennifer Felker, Megan Mazzotta, Andrea Mendez Colmenares, Jules Skoda, Kathy Keefer, Augusto Miravalle

A comprehensive approach to patient understanding of brain health in Multiple Sclerosis (MS) is an unmet need. The Brain Health Program utilized a flipped classroom model to provide educational tools to patients with MS. The flipped classroom model allows learners to encounter information before class, freeing class time for higher-order thinking and inquisition. To date, there is no known use of this approach in patient education. Improvements in healthcare literacy were assessed in 24 individuals with MS who participated in the Brain Health Program. Demographics include age, sex, race and disease duration. All participants completed 16 pre-recorded online modules on topics including disease-modifying therapies, fatigue, cognition, depression/anxiety, living with MS/caregiver support, family planning, social security disability and employment, new therapies, exercise, physical therapy, occupational therapy, sleep, and nutrition. After completion of these modules, patients had access to 6 live virtual 1-hour sessions. Each session had a balance between didactic, experiential learning and a Q&A portion. At baseline, and after the completion of the program all participants completed a brief healthcare literacy in MS questionnaire. 71% of participants improved their understanding of MS to make educated decisions on their MS therapies, 67% felt more confident in their knowledge of MS, and 92% felt more familiar with the concept of brain reserve, brain health, and the difference between MS progression and deconditioning. 100% of participants rated each session favorably and up to 90% of participants indicated that they felt more empowered to live with MS as a result of what they learned. These results support the Brain Health Program flipped classroom model as an effective method to improve health care literacy in patients living with MS. Moving forward, it is important to fine-tune the modules to derive the most benefit and maximize patient learning and empowerment.

Medical Student/Other

O-37. Stereotactic body radiation therapy for oral tumors in canine patients.

Patricia Gualtieri, Ber-In Lee, Tiffany Martin, Del Leary, Susan M. LaRue, Mary-Keara Boss

Purpose/objective: To describe outcome and toxicity for dogs with oral tumors, specifically malignant melanoma (OMM), squamous cell carcinoma (SCC) and soft tissue sarcoma (STS) following stereotactic body radiation therapy (SBRT). Materials/methods: A single institution retrospective study was conducted of dogs with macroscopic OMM, SCC and STS treated with SBRT. Patient signalment, clinical characteristics, treatment parameters and tumor responses were recorded. Overall progression-free survival (PFS), local PFS and overall median survival time (MST) were calculated using Kaplan–Meier analysis. Acute and late toxicity were recorded according to VRTOG criteria. Adverse events were recorded. Results: A total of 98 patients met the inclusion criteria (OMM n = 37, SCC n = 18, STS = 43). The SBRT prescription was given over 1-6 fractions, with a total dose range of 12-40 Gy. Overall PFS was 152 days and MST was 270 days, with no statistical difference between tumor types (p value = 0.2 and 0.4). Local PFS for OMM, SCC and STS was 187, 253 and 165 days, respectively. Acute toxicities affecting skin, mucosa, eyes and/or CNS occurred in 42/85 patients (49.4%), with VRTOG grade 3 affecting 10/85 (11.7%) of patients. Late toxicities affecting skin, bone, joint and/or eyes occurred in 35/80 (43.7%) of cases that were alive at 3+ months, with VRTOG grade 3 affecting 24/80 (30%) of patients. Prognostic factors for response, outcome and toxicity are analyzed. Conclusions: SBRT can be offered as a treatment option for oral tumors in dogs. Toxicities were common and warrant risk factors considerations as well as appropriate veterinary and client education.

Graduate Student/ CS

O-38. Radiographic appearance of the cecum in dogs

Stephanie Stromberg, Hock Gan Heng, Lauren Nakonechny, Tiffany Martin, Craig Webb, Elissa Randall

Although the canine cecum is commonly identified on abdominal radiographs, a detailed description of its appearance is rare. There is no literature with the specific focus of describing its position, size, shape, or contents, leading to different opinions of what is considered normal. Therefore, the aims of this study were to 1) report how frequently the cecum can be identified, 2) characterize the typical location, contents, size, and shape of the cecum, and 3) provide a preliminary comparison of these features between dogs with and without gastrointestinal signs. All abdominal radiographic studies acquired from September through November 2022 were retrospectively evaluated. The cecum was identified in at least one view in 110/185 studies (59%) which met inclusion criteria. When identified, the cecum always contained gas (100%), frequently contained feces-like content (37%), and rarely contained fluid (4%). The greatest diameter of the cecum ranged from 2.5cm - 15.4cm (mean 7.2cm in the VD view), and it was most often coiled in shape (68%). The cecum was usually located within the mid or dorsal abdomen in lateral projections and the mid or right abdomen in VD projections. It was most commonly at the level of the T13-L5 vertebrae, occasionally identified as far cranial as T11 or caudal as L7. No significant differences were identified in any evaluated parameter of cecal size, shape, content, or location between dogs with and without GI signs. This study will help to unify our understanding of the radiographic appearance of the cecum, and may provide a basis of comparison for future investigations of cecal pathology.

Resident/ERHS

O-39. Evaluating the confidence and competency of leadership, teamwork, and communication skills in veterinary students during the RECOVER CPR training course

Claire Tucker, Ilana Weisburg, Lauren Rush, M. Travis Maynard, Julien Guillaumin, Kristin Zersen, Kelly Hall

This study evaluated veterinary students at Colorado State University for changes in confidence and competence in leadership, teamwork, and communication after participation the RECOVER Cardiopulmonary (CPR) Advanced Life Support (ALS) training. Veterinary students receive intensive communication training, mostly focused on general practice and client-facing scenarios. While this process is highly successful in teaching communication in a non-emergent setting, there is a lack of instruction in emergency team communication skills. This study evaluated RECOVER training, a CPR lab using high-fidelity simulators, as a tool to fill that gap. The study's first objective was to measure growth in individual confidence with respect to teamwork, leadership, and communication skills during RECOVER ALS. The study's second objective was to measure growth in competence in the same setting. The study evaluated the changes in confidence levels of fourth year veterinary students via self-reported scores on a Likert-scale and competence via a trained observer evaluating specific communication objectives. The persistence of confidence and competence over time was assessed by re-evaluating the learners at 2 and 6 months following the initial training. Nonparametric ordinal data was analyzed using Mann-Whitney Test and Wilcoxon Signed-Rank Test. All measures of confidence increased from pre-survey to post-survey (pooled median Likert 3.4 to 4.7). Confidence and competence at the re-evaluations decayed from the first assessment but remained significantly higher than levels prior to the intervention. Competency of a team was significantly linked to the combined confidence measurements of team members and whether the team had all the same members participating in the scenario. This study demonstrates that high-fidelity simulation RECOVER ALS training is an effective tool for the delivery of key competencies for emergency clinicians, but confidence and competence requires deliberate practice and consistent team members. This study will help guide training for clinicians to lead teams in emergent settings. Research funded by American College of Veterinary Emergency and Critical Care RECOVER Grant.

Resident/ CS

O-40. Solutions to Enhance Health with Alternative Treatments (SEHAT): a double-blinded randomised controlled trial in Indonesia for treatment of severe acute malnutrition using rice bran in ready-to-use therapeutic foods (RUTF)

Annika M. Weber , Silvia Barbazza, Moretta D. Fauzi, Asrinisa Rachmadewi, Ririh Zuhrina, Fildzah K. Putri, Maiza Campos-Ponce, Marinka van der Hoeven, Rimbawan Rimbawan, Zuraidah Nasution, Puspo E. Giriwono, Frank T. Wieringa, Damayanti D. Soekarjo, Eliza

Current formulations of ready-to-use therapeutic foods (RUTFs) to treat severe acute malnutrition (SAM) in children focus on nutrient density and quantity. Less attention is provided to foods targeting gut microbiota metabolism. Heat-stabilized rice bran contains essential nutrients, prebiotics, vitamins and unique phytochemicals that have demonstrated favorable bioactivity to modulate gut microbiota composition and mucosal immunity. This study seeks to examine the impact of RUTF with rice bran on the microbiota during SAM treatment, recovery and post-treatment growth outcomes in Jember, Indonesia. A total of 200 children aged 6-59 months with uncomplicated SAM [weight-for-height z-scores (WHZ) < -3, or mid-upper arm circumference (MUAC) <115mm or bilateral pitting oedema +/++] or approaching SAM (WHZ < -2.5) were enrolled in a double-blinded, randomized controlled trial. Children in the active control arm received a locally produced RUTF; those in the intervention arm received the local RUTF with 5% rice bran. Among the children enrolled, still blinded for analysis, 38.5% of were 6 to 23 months old, 42% were female, baseline median WHZ was -2.97 (-3.27, -2.78), and baseline median MUAC was 12.80 (12.40, 13.30) cm. After treatment six children in RUTF A and two children in RUTF B arm recovered from malnutrition. Primary outcomes of changes in weight, growth z-scores, MUAC, and morbidity, as well as gut microbiome modulations will also be assessed as an intention-to-treat analysis. Findings are expected to provide novel mechanisms for rice bran prebiotics with functional bioactivity to improve SAM treatment outcomes. Study registered on clinicalstrails.gov (NCT05319717). Research funded by the Thrasher Research Fund.

Graduate Student/ERHS

O-41. Immune responses to radiation therapy and immunomodulation in an oral squamous cell carcinoma mouse model

Amber Beeney, Braden Burdekin, Nolan Sweeting, Ber-In Lee, Mary-Keara Boss

Purpose: New therapies are needed for advanced oral squamous cell carcinoma (SCC). Propranolol (P) and Losartan (L) are repurposed immunomodulatory drugs which decrease immunosuppressive myeloid cells. L also improves tumor oxygenation. Our overall hypothesis is that P+L and radiation (RT) will improve survival in a syngeneic, orthotopic oral SCC mouse model compared to RT alone due to immunomodulatory and vascular effects. Methods: Murine LY2 SCC was injected into the buccal mucosa of BALB/c mice. Two days later, mice were randomized to treatment (n=6/group) with daily intraperitoneal (IP) injections of either saline control (C), L, or P, and two groups received radiation therapy (RT) (4Gy x 3; L+RT, P+RT) when tumor volumes reached 150 mm3. Body weight and tumor volumes were monitored until morbidity endpoint. Kaplan-Meier survival curves were generated and compared via log-rank test. Results: There was a statistically significant difference in survival times across the treatment groups. Neither L nor P alone improved survival compared to C, but mice treated with either L+RT or P+RT lived significantly longer than C, L, and P groups. There was no survival difference between L+RT or P+RT. Conclusion: RT improved survival when combined with P or L compared to other groups. These results will be integrated into a larger mouse study investigating C, PL, RT, RT+PL. Serum cytokine analysis and pathologic review and immunohistochemical quantification of immune and endothelial cells from tissue samples are underway. Research reported in this poster was supported by K01OD031809 (Boss). Student support (A Beeney) was provided by NIH Training Grant T35OD015130.

DVM Student/ BMS

O-42. Elucidating Polymorphisms in *Plasmodium falciparum* from Asymptomatic Malarial Samples in Homa Bay Residents

Jebrail Dempsey, Elizabeth Hemming-Schroeder

Malaria remains a leading public health threat in sub-Saharan Africa, specifically Kenya and Ethiopia. *Plasmodium falciparum*, a common species of malaria-causing parasites in sub-Saharan Africa, is spread through mosquito bites. Finding effective strategies in treating and preventing the spread of malaria is difficult due to the variability in the parasite's genome. Intervention techniques, such as insecticide-treated bed nets, have decreased malaria transmission; however, these materials cannot stop the emergence of hotspots, or regions where cases remain high as transmission decreases. While there is one WHO-approved malaria vaccine – RTS,S – its long-term effects remain to be seen. Detection of low parasitemia infections may provide better chances of treating malaria before it spreads. Genetic variation within malaria infections confers drug resistance, immune evasion and severe disease. Genotyping *P. falciparum* samples may provide insight into the dynamics of malaria infections. We propose identifying microhaplotypes of *P. falciparum* to better detect asymptomatic cases of malaria that fuel these hotspots. Using selective whole genome amplification (sWGA) and highly-multiplexed amplicon sequencing, we can detect and magnify parasite DNA from infected blood then identify polymorphisms of interest. Alongside our biological data, we also have survey data, allowing us to observe transmission trends and their relationship to environmental and lifestyle factors. Other studies have shown that hotspots with low transmission levels may have higher genetic variation. As previously described in the literature, we expect to see heterogeneous expression in the var gene from the parasites we are sampling. Our results may inform us of future, potential therapeutic targets. A persistent issue in treating malaria is the multitude of mutations that prevent the efficacy of diagnostic tests and drugs. We plan to include other socioeconomic and environmental factors in our survey analysis, such as land use, access to clean and potable water, and trends of extreme weather. Student support provided by NIH training grant T32GM144856.

Graduate Student/MIP

O-43. Neuroinflammatory and accumulation of misfolded proteins as biomarkers in canine cognitive decline

Kassandra Oldham, Masa Ukai, Tatum Flatt, Breonna Kusick, Stephanie McGrath and Julie A. Moreno

Canine cognitive dysfunction syndrome (CCD) is a naturally occurring progressive neurodegenerative disease that commonly affects geriatric dogs, with age being the number one risk factor. Similar to humans with Alzheimer's disease (AD), it is known that the canine population has similar pathologic changes, yet the overall pathogenesis of CCD is poorly understood. Two characteristic features of AD are the post-mortem presence of neurofibrillary tangles (NFTs), composed of abnormally phosphorylated tau (P-tau), and the deposition and accumulation of the protein beta amyloid (Aβ), forming plaques. These extracellular depositions prevent the proper function of neuronal cells, eventually leading to cell ischemia and cell death. Increases in glial inflammation accompany the Aβ and NFTs, causing tissue damage and contributing to the clinical signs seen with AD. Recent studies of post-mortem aging canine brains have demonstrated an increase in glial inflammation, accumulation of Aβ, and tau phosphorylation (P-tau).. The only current antemortem diagnostic for CCD is owner questionnaires combined with veterinary physical and neurological examinations. This study aimed to investigate P-tau, Aβ1-42 aggregation, increased microglial number and activated astrocytes as possible biomarkers of CCD onset and prognosis. While diligently analyzing additional canines for statistical significance, preliminary results show a trending increase in proteins of CCD positive canines in all three groups of interest, specifically Aβ1-42, S100β (astrocyte marker for neuroinflammation), and T217 (abnormal phosphorylation site on tau protein). Utilizing a cohort of presumptively diagnosed CCD positive and a cohort of CCD negative geriatric canines, we investigated these neurodegenerative proteins via immunohistochemistry staining and microscopy image analysis. Research support provided by Institute of Cannabis Research, Panacea Life Studies and Dog Aging Project. Student support provided by NIH training grant T35OD015130.

DVM Student/ ERHS

O-44. Modeling the Effects of an Emerging Toxicant, Wildfire Smoke, on Reproductive Toxicity Using *Caenorhabditis elegans*

Jacob Smoot, Abdullatif Alsulami, Julie Moreno, Luke Montrose

Intensifying wildfires, driven by hotter and drier climates, subject us to increased smoke exposures year over year. While a large body of literature detailing cardiopulmonary effects of these inhaled toxicants exists, new evidence suggests that the reach of these pollutants extends to the reproductive system. This poses significant public health burdens, specifically on pregnant persons, who may face having their children suffer from lower birth weight, preterm birth, and birth defects due to smoke exposure. By screening for reproductive toxicity using the *C. elegans* model, we can understand the reproductive risks associated with exposure to wildfire smoke (WFS). Simulated WFS from Douglas Fir needles was generated in a combustion chamber at 500C and collected onto PTFE filters, then extracted into a solution for experimentation. *C. elegans* were exposed to WFS at 200 or 1000 μg/ml to assess general toxicity and the impacts of WFS upon progeny and their viability. The total number of eggs, as well as F1 worms, were counted at 24, 48, 72, and 96 hours after plating. Similarly exposed *C. elegans* will be assessed for germ cell apoptosis using fluorescent microscopy analysis to assess the mechanism of WFS on reproductive toxicity. At 24-, 48-, and 72-hours past plating, no significant changes in progeny viability were seen from the egg hatching tally. However, at 96 hours past plating, both treatment groups differed significantly from control and from each other. Exposure to WFS at 200 and 1000 μg/ml significantly lowered total *C. elegans* F1 counts ($p = 0.0347$, 0.0242, respectively). Worms treated with 1000 μg/ml also had significantly lower F1 counts compared to worms treated with 200 μg/ml ($p = 0.0077$). Our studies thus far demonstrate that exposure to WFS results in reproductive toxicity in C. elegans though the mechanism remains to be elucidated.

Graduate Student/ERHS

O-45. Omega-3 fatty acid metabolite Resolvin D1 assists recovery after agricultural dust exposure

Alissa N. Threatt, Logan S. Dean, Melea Barahona, Carly S. Chesterman, Kaylee Jones, Emmanuel Oyewole, Maelis Wahl, Ash Ibarra, Tara M. Nordgren

Organic dust exposure causes chronic pulmonary diseases in agriculture workers. These diseases have few treatments with limited efficacy; however, omega-3 (ω-3) fatty acids and their metabolites, such as Resolvin D1 (RvD1), have been shown to aid in recovery from lung injury. Mouse alveolar macrophages (MH-S line) were cultured and stimulated with RvD1 with or without organic dust extract (DE). Supernate and cells were collected over 24 hours to evaluate inflammatory mediator production at protein and transcript levels. C57BL/6 (WT) and il22-/- (KO) mice were intranasally (IN) instilled with saline or DE 5 days per week for 3 weeks and treated with RvD1 intraperitoneally (IP) once weekly. Animals were sacrificed 5 hours or 3 days post-last instillation. Bronchoalveolar lavage fluid (BALF) was collected for cytokine and cell infiltrate analyses and lungs were harvested for histopathology and transcript evaluation. MH-S cells co-exposed with DE and RvD1 exhibited decreased production of pro-inflammatory mediators and increased production of pro-resolution mediators. Animal studies revealed increased immune cell infiltration in dust-exposed animals with altered cell infiltration in RvD1-treated animals. Decreased pro-inflammatory cytokines were also observed in dust-exposed KO animals treated with RvD1 with a 3-day recovery compared to WT and saline controls. Histopathology revealed increased lymphoid aggregates, alveolar inflammation, and peribronchiolar inflammation in DE-exposed animals with decreased lymphoid aggregates in both animals exposed to DE and treated with RvD1 with a 3-day recovery. These data suggest RvD1 resolves inflammation and initiates repair pathways more rapidly and implicates its utility in possible therapeutic applications. Research support provided by NIH/NHLBI R01HL185926 to TMN.

Graduate Student/ ERHS

O-46. Implantation and early placentation in the mare: the role of kisspeptins in trophoblast invasion

Ryan Eastman, Sam Fisher, Lauren Young, Linda Lott, Jeremy Cantlon, Rick McCosh, Christianne Magee

The invasion of trophoblast cells and subsequent formation of endometrial cups is a crucial step in equine placental development. Yet, much remains to be elucidated about this process in the context of kisspeptins. The structure of the human placenta has many similarities to the horse, kisspeptins and their receptor have been shown to regulate invasion of the trophoblast cells as they penetrate the maternal endometrium. During this intrusive process, maternal and fetal communication is key in producing a successful implantation with studies from humans showing that a dip in kisspeptin concentrations to be correlated with complications such as intrauterine growth restriction during early pregnancy. Using immunohistochemistry, samples of biopsies of the fetal-maternal interface and uterus from 6 pregnant mares at day(d) 30, d36 and d40 (n=2/day, ovulation d0), and 2 non-pregnant mares were stained for kisspeptin, Kiss1r, a trophoblast marker, and progesterone receptor. Preliminary results suggest that colocalization of kisspeptin and its receptor, Kiss1r, can be observed in mares that have undergone the trophoblast invasion stage, suggesting that this interaction is present during this phase. Expression of kiss1 and Kiss1r using ddPCR in can be observed in nonpregnant and pregnant mares, but the greatest expression is found in the pregnant horn adjacent to the implantation site. Additional samples of isolated fetal and maternal tissues are being analyzed to better understand the role of kisspeptin in trophoblast cells invasion and thus the formation of endometrial cups. Research support provided by the Grayson Jockey Club Research Foundation.

Graduate Student/BMS

O-47. Do equine stromal cells maintain pre-injury mobility in a mouse osteoarthritis model?

Heidi Kloser, Parvathy Thampi, Taryn Boxleitner, Nyzek Rodriguez, Brian Johnstone, Katie Sikes, Kelly Santangelo, Laurie Goodrich

Stromal/stem cell therapies have been pursued heavily as potential interventions for osteoarthritis (OA). Traditionally, bone-marrow-derived mesenchymal stromal cells (MSCs) have been utilized for these approaches; however, their beneficial effects are often short-lived. Articular ChondroProgenitors (ACPs), a type of stem cell found in the articular cartilage of joints, can retain their cartilage-forming ability after many expansions. In this study, we investigated the effects of these longer-lasting ACPs vs. the standard MSC therapy's protection against OA after a knee injury in a mouse model. Cell therapies for this study were generated from three horses in parallel. MSCs were processed from bone marrow and APCs from cartilage punches. Cells were evaluated in culture for their anti-inflammatory properties. OA was induced by a destabilization of the medial meniscus (DMM) injury; MSCs, ACPs, or vehicle control were injected into joints 1-week post-injury. Mice were monitored for behavior/mobility pre-injury and at weeks 2, 4, and 8 of the study. Upon euthanasia, the injured joint was collected for histological scoring of OA. In vitro, results confirmed the anti-inflammatory abilities of MSCs and ACPs compared to control groups. Two weeks after injury, mice receiving either cell treatment had statistically significant differences in voluntary movement and speed compared to the controls. At week 8 post-injury, the treated animals spent significantly more time moving, less time in their hut, and had fewer freezing episodes. The treated animals also had more consistent hindlimb gait parameters relative to baseline at week 8 than the controls. Histological scoring performed to date showed near significant differences between the two stem cell treatments, with ACPs having less aggressive OA than MSCs. The mobility and gait data indicate that both MSCs and ACPs improved animal mobility. Results from joint scoring indicate that ACPs may provide better protection against OA long-term. Research support provided by PPF-2021-101639 and NIH training grant T32AI162691.

Graduate Student/ MIP

O-48. Primary lung fibroblasts promote therapy resistance in co-cultured triple negative breast cancer cells through a juxtacrine, JAK-dependent signaling mechanism.

Marika Klosowski, Claire Stratton, Qiong Zhou, Hector Esquer, Daniel LaBarbera, Daniel Regan.

Triple negative breast cancer (TNBC) carries a poorer prognosis than other breast cancer subtypes due to high metastatic incidence, lack of molecular drug targets, and rapid development of chemoresistance. Cancer associated fibroblasts are key extrinsic drivers of chemoresistance in primary TNBC tumors, but it is unknown whether resident fibroblasts in distant metastatic tissues such as the lung also influence TNBC drug sensitivity. We previously developed a breast cancer cell (BCC) - primary normal lung fibroblast (LF) *in vitro* lung metastasis model using the TNBC cell line MDA-MB-231 (MB231) and applied this model in a high throughput screen of 846 kinase inhibitor compounds with the goal of uncovering mechanisms of LF-mediated chemoresistance and therapeutic vulnerabilities relevant to lung-metastatic TNBC. This drug screen identified reproducible differential MB231 responses to kinase inhibitors between monoculture and co-culture conditions. When co-cultured with lung fibroblasts, MB231 cells became broadly more resistant to kinase inhibitor compounds but showed increased sensitivity to inhibitors of the lipid kinase VPS34, an important initiator of autophagy. Using a flow cytometric assay for autophagy quantification and drug combination assays, we established that autophagy is increased in co-cultured TNBC cells and contributes to resistance to MCB-613 and ponatinib, two compounds identified in our drug screen. We found that neutralizing IL-6, a cytokine highly secreted by LFs in co-culture, or adding IL-6 to monocultured MB231 cells did not significantly alter their autophagic flux or therapy responses, though inhibition of downstream Janus kinase (JAK) signaling prevented LF-mediated increases in autophagy and therapy response alterations in co-cultured MB231 cells. Rather, using transwell cell culture assays, we determined that cell-cell contact is required for LF modulation of MB231 therapy responses in co-culture. Thus, we concluded that LFs enhance autophagy and therapy resistance in co-cultured MB231 cells through a JAK-dependent juxtacrine signaling mechanism. The work for this project was supported by NIH grants K01OD022982 (Dan Regan), U01 COHA Translational Research Fellowship (Marika Klosowski), NIH Training Grant T35OD015130 (to Claire Stratton), and P30 CA046934 (University of Colorado Cancer Center Support Grant).

Post-doctoral Fellow/MIP

O-49. Characterization of air pollutants at equine racetracks in the United States from 2011-2020

Kimberly Kreitner, Danni Scott, Linda Kim, Katie Seabaugh, Colleen Duncan, Sheryl Magzamen

Little is known about the impact of smoke and air pollution on animal athletes. The purpose of our study was to characterize air quality near thoroughbred racetracks and determine if they are at an elevated risk of air pollution. To assess air quality near racetracks, we acquired data from the United States Environmental Protection Agency for the following air pollutants: particulate matter 2.5 and 10 microns ($PM_{2.5}$, PM_{10}, respectively), ozone, NO_2, CO, SO_2, and lead . We assessed air quality data within a 50km and a 100km radius surrounding each track in the United States from 2011 to 2020. To assess the daily pollutant concentration at each racetrack, we took the average of every air quality monitor within each radius. We identified 66 racetracks that had air quality monitors within a 50km radius. All criteria pollutants had at least one racetrack that surpassed the daily recommended threshold except for CO. Ozone had the most daily threshold violations within the 50km radius (n= 2094), followed by lead (n= 1849), $PM_{2.5}$ (n=615), PM_{10} (n= 136), SO_2 (n= 65), and NO_2 (n = 1). The air quality monitors are representative of possible air quality near the racetracks, however actual levels of pollutants may vary, particularly for pollutants whose levels may vary further from the monitor, such as lead. Future studies should evaluate air quality specifically at the racetrack locations, on race days to better elucidate the impact of air pollution on race times and health of equine athletes. Research support provided by a CVMBS CRC award.

Graduate Student/ ERHS

O-50. Single-cell RNA sequencing analysis of the duodenal mucosa in dogs with chronic inflammatory enteropathy

Allison Manchester, Dylan Ammons, Michael Lappin, Steven Dow

Chronic inflammatory enteropathy (CIE) is a common condition in dogs causing recurrent or persistent gastrointestinal clinical signs. Pathogenesis is thought to involve intestinal mucosal inflammatory infiltrates, but histopathological evaluation does not predict treatment response, inform prognosis, or correlate with clinical remission. We employed single-cell RNA sequencing to catalog and compare the diversity of cells present in duodenal mucosal endoscopic biopsies from healthy dogs (n=3) and dogs with CIE (n=4). Through characterization of 35,668 cells, we identified 30 transcriptomically distinct cell populations, including epithelial cells, T cells, myeloid cells, stromal cells, and plasma cells. Both healthy and CIE samples contributed to each cell population. Differences were noted in prevalence of T cell subpopulations, with CIE samples showing a relative decrease in resident memory and increase in infiltrative T cells. Neutrophils from CIE samples were qualitatively more common and appeared more inflammatory, expressing greater amounts of SOD2 and IL1B compared to healthy. One subset of epithelial cells from CIE dogs showed differential expression of a gene encoding a 2-pore potassium channel (KCNK16). Overall, this work reveals a previously unappreciated cellular heterogeneity in canine duodenal mucosa and provides insights into molecular mechanisms to enhance understanding of CIE. The cell signatures developed through this study can be used to deconvolute bulk RNA-seq data in the future to allow more accessible interrogation of cellular activities in health and disease. Research support provided by the Center for Companion Animal Studies, Nestle Purina, and Immunotherapy Research Laboratory.

Post-doctoral Fellow/CS

O-51. The cell of origin of canine CD4+ peripheral T-cell lymphoma

Eileen Owens, Adam Harris, Anne Avery

Peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) is a malignancy in humans and dogs with poor survival times and treatment responses. The cell of origin of PTCL-NOS remains unclear, which hinders our understanding of its pathogenesis and appropriate treatment. To address this, we compared the gene expression profiles of canine CD4+ PTCL to normal nodal CD4+ T cells and CD4+ thymocytes. Bulk RNA-seq was performed on 96 canine nodal CD4+ PTCLs diagnosed by flow cytometry and sorted CD4+ nodal lymphocytes and CD4+ thymocytes from healthy control dogs. Raw reads were aligned to the canine genome (ROS_Cfam_1.0) and tabulated. Post data normalization and differential gene expression analysis were conducted with DESeq2, and differentially expressed genes were compared to various stages of T-cell development via gene set enrichment analyses. Statistical significance was defined as an adjusted p-value ≤0.05. Canine CD4+ PTCL had increased expression of *GATA3* (log2fc=1.9), a canonical T cell transcription factor in human PTCL-NOS, and genes of immaturity, including *CD34* (log2fc=7.8), *KIT* (log2fc=3.3), and *CCR9* (log2fc=4.8), although surface CD34 expression is not detected by flow cytometry. Additionally, canine PTCL-NOS samples were enriched for human and murine early thymocyte progenitor cell gene signatures. These findings were further supported when we compared the canine CD4+ PTCL gene expression programs to a canine single cell transcriptomic atlas of hematopoietic precursors and T cells across canine bone marrow, thymic, and lymph node tissues. Canine CD4+ PTCL was significantly enriched for gene signatures associated with early thymic and bone marrow precursors (NES 1.3-2.1), and negatively enriched for gene signatures associated with naïve nodal CD4+ T cells (NES=-3.2). In conclusion, the gene expression profile of canine CD4+ PTCL resembles the GATA3-PTCL subtype of human PTCL-NOS, and a subset of these neoplasms may arise from a thymic precursor cell of origin. This work was supported by NIH training grant T32OD010437 and the Clinical Hematopathology Laboratory at Colorado State University.

Post-doctoral Fellow/ MIP

O-52. Sex differences in intestinal Parkinson's Disease pathology

Hayley Templeton, Alexis Ehrlich, Luke Schwerdtfeger, Stuart Tobet

Parkinson's Disease (PD) is a neurodegenerative disorder with gastrointestinal (GI) symptoms such as constipation and visceral pain, often emerging decades before motor issues. The pathological hallmark of PD is accumulation of misfolded α-synuclein (aSyn) protein in the brain and periphery, including the GI tract. Men have two times the risk of developing PD compared to women. However, disease progression and mortality are higher in women, who also report worse GI symptoms. Gut calcitonin gene related peptide (CGRP) is involved in modulating intestinal contractions and pain. We hypothesize that sex differences in GI symptoms of PD are the result of sex specific aSyn aggregation and differential enteric CGRP signaling. Intraperitoneal injections of the pesticide rotenone were given to C57BL/6J mice to facilitate aggregation of aSyn. Immunohistochemistry was performed on colon. Vehicle treated females had 48% (0.66 μm2) less immunoreactive (ir) enteric aSyn than males (1.3 μm2). Rotenone treatment increased aSyn-ir by 56% (1.5 μm2) in females and 28% (1.08 μm2) in males. Rotenone treatment increased the average percent area of CGRP-ir in the apical crypt (male 9% +/- 0.6; female 10%) compared to vehicle (male 7% female 7%). This increase was 14% higher in females compared to males. The larger increase in apical CGRP-ir after rotenone treatment in females may contribute to sensitivity to noxious stimuli from the lumen. This finding coupled with larger aSyn increases in females provide valuable data towards explaining increased GI symptoms seen in PD females. Females are significantly understudied within the context of PD, however, investigating sex differences in the underlying pathophysiology of GI symptoms could provide clues to sex differences seen in clinical outcomes. Since these symptoms often arise years before motor dysfunction and diagnosis, it is critical to determine sex specific mechanisms of aSyn aggregation and influence on enteric neural involvement in GI symptoms.

Graduate Student/BMS

O-53. Gut microbiome dysregulation is associated with sustained inflammation and differential gene expression in equine progressive osteoarthritis

Zoë J. Williams, Lyndah Chow, Gabriella Piquini, Ashana Patel, Meagan Rockow, Steven Dow, Lynn Pezzanite

Osteoarthritis (OA) represents one of the most common disorders treated in equine practice, leading to joint degeneration, pain, and disability. Although many factors contribute to OA development, the gut microbiome has recently emerged as an important pathogenic factor in OA initiation and progression in humans, which has not been previously investigated in horses. The objective of this study was to compare microbial populations in feces and circulating peripheral blood mononuclear cells (PBMC) in horses with and without OA and correlate those findings to transcriptomic analyses of synovial fluid (SF) cells and cytokine levels in plasma and SF in the same study population. SF, peripheral blood, and feces were collected from 18 horses (n=12 healthy, n=6 OA). DNA was extracted from feces and PBMC and 16s sequencing performed for bacterial DNA. RNA was extracted from SF cells and PBMC and sequenced via an Illumina-based platform. Principle coordinate analysis using Bray-Curtis dissimilarity demonstrated differences in beta diversity quantification in overall taxonomic composition between feces ($p=0.003$) and PBMC ($p=0.01$) from horses with and without OA, with a relative increase in Firmicutes phyla abundance in OA. Specific bacterial phyla (*Firmicutes* predominantly, *Verrucomicrobia*, *Tenericutes*, *Fibrobacteres*) correlated to transcriptomic differences related to cell structure, extracellular matrix, and immune response to inflammation in OA. Microbial and transcriptomic differences in OA correlated to elevated inflammatory biomarkers in SF (IL-1ß ($p=0.02$), IL-6 ($p=0.005$), G-CSF ($p=0.02$)) and plasma (IFN-γ ($p=0.004$), IL-18 ($p=0.0007$), IL-6 ($p=0.02$), IP-10 ($p=0.02$)) in OA. These findings indicate microbial dysregulation may play a role in OA progression in horses. Similarities noted in bacterial phyla altered in horses with OA vs. humans further indicate horses may represent a relevant preclinical model to investigate the gut-joint-axis of OA across species. Future studies will investigate avenues for treatment directed towards microbiome dysregulation and systemic inflammation versus previously joint-centric approaches. This work was supported by Animal Health and Disease Grant No. NI22AHDRXXXXG011 Project Accession No. 7003828 from the USDA National Institute of Food and Agriculture.

Post-doctoral Fellow/ CS

1. Novel extractants for lead (II) liquid-liquid separations

Nicole Ahrens, John Despotopulos, Ralf Sudowe

Current liquid-liquid extractants are incapable of achieving kinetics favorable for separations of some of the heaviest transactinides, with half-lives on the order of a few seconds. Element 114, flerovium, is a recent example of a very short-lived (289Fl, t1/2 = 2.1s) transactinide for which the chemical characteristics in the liquid phase have yet to be studied due in part to the lack of a suitable liquid-liquid extraction system. Liquid-liquid studies were performed using 212Pb (t1/2 = 10.62 h), a group 14 homolog of Fl, utilizing the novel extractant hexathia-18-crown-6 paired with ionic liquid solvents co-dissolved in carbon tetrachloride and evaluated for extraction efficiency and kinetics. Favorable extraction kinetics are expected to help enable some of the first aqueous-phase studies of transactinides with Z > 106. This research was support by U.S. Nuclear Regulatory Commission award 5331310021M0028.

Graduate Student/ ERHS

2. CXCR2 perturbation promotes Staphylococcus aureus implant-associated infection

Mike Akaraphanth and Casey Gries

Staphylococcus aureus is the leading cause of acute medical implant infections in humans. Its success resides in its virulence factors, resistance to numerous antimicrobials, mechanisms of immune modulation, and ability to rapidly form biofilms associated with implant surfaces. Biofilm-mediated infections are notoriously difficult to treat, and dissemination from the biofilm can result in life-threatening bacteremia. Thus, S. aureus device-associated infections are often persistent, skewing innate immune responses to promote chronic recurrent infections. Immune effector cells must be efficiently recruited to sites of infection via directed chemotaxis, however, little is known of the role neutrophils play in response to acute S. aureus biofilm infections. Here we investigate the role of CXC chemokine receptor 2 (CXCR2), predominantly expressed on neutrophils, during murine S. aureus implant-associated infection. We hypothesize that modulation of CXCR2 expression and/or signaling during S. aureus infection, and thus neutrophil recruitment, extravasation, and antimicrobial activity, will affect infection control and bacterial burdens in an implant-associated infection model. To study the role of CXCR2 during S. aureus implant infection, we infected indwelling subcutaneous catheters with a community-associated methicillin-resistant S. aureus strain. Treatment groups received daily intraperitoneal doses of Lipocalin-2 (Lcn2) or AZD5069 to assess CXCR2 induction or inhibition, respectively. At study conclusion, we analyzed catheters and surrounding tissues for bacterial burdens and dissemination, and quantified Cxcr2 transcription within implant-associated tissues. Mice treated with Lcn2 resulted in higher bacterial burdens and increased Cxcr2 expression within the soft tissues. AZD5069 treatment also resulted in increased implant- and tissues-associated bacterial titers, with enhanced Cxcr2 expression. Our results demonstrate that CXCR2 plays an essential role in regulating the severity of S. aureus biofilm-mediated infections; perturbation of expression or signaling both resulted in elevated implant-associated bacterial burdens. Thus, CXCR2 appears finely tuned to efficiently recruit effector cells and mediate control of S. aureus biofilm-mediated infection. This work was supported by a CVMBS CRC award.

Medical Student/MIP

3. Developing methods to sample potential resuspension of radioactive contaminants near the former Rocky Flats Technical Plant

Richard V. Alcantar, Ralf Sudowe

From 1952 until 1989, the Rocky Flats Technical Plant processed plutonium for use as triggers in nuclear weapons. Throughout the facility's nearly 37 years in operation, several events led to radioactive contamination in areas within and surrounding the site. Since then, multiple cleanup projects have occurred, remediating contamination to acceptable levels. However, an increase in the number, size, and severity of Colorado wildfires in recent years has raised public concern for the potential resuspension of radioactive surface contamination to the now-populous areas surrounding Rocky Flats. Air sampling during specific conditions such as high winds, naturally occurring wildfires, and controlled burns would provide valuable data to determine if resuspension of radioactive contamination may be of concern. Sampling under such circumstances, however, is restricted by situation, permission, and weather. Whereas traditional aerosol sampling collects "total dust" samples to amass particles in the air with equal efficiency, without regard to particle size fraction, the use of a cascade impactor to separate aerosols by size can be utilized to relate how deep varied-sized particles might penetrate the human respiratory tract after inhalation. This would not only indicate the presence of radionuclides but also the deposition location within the human body, an important factor in determining the best dose estimate for the person. This study will determine if a 3D-printed cascade impactor can provide air sampling data comparable to a commercial cascade impactor. Conclusive data will result in an easily accessible air sampling method to determine the presence of aerosolized or resuspended contaminants and possible deposition location within the human respiratory tract.

Graduate Student/ ERHS

4. Cellular Stress Responses Following Wildfire Smoke Exposure in *Caenorhabditis elegans*

Abdullatif Alsulami, Jacob Smoot, Ava Hozler, Luke Montrose, and Julie A. Moreno

Environmental stressors, such as wildfire smoke (WFS) and reduced air quality, threaten global health and risk for exposure is increasing with climate change. WFS is composed of a variety of chemicals with particulate matter (PM) being the highest weight by volume constituent. PM with an aerodynamic diameter of 2.5 μm (PM2.5) causes pulmonary damage and more recently is linked to harmful extrapulmonary effects, including the central nervous system. Epidemiological studies have associated PM2.5 exposure with an increased risk of cognitive impairment as well as increases in neuroinflammatory responses. However, the damage caused by WFS-derived PM2.5 on the brain is not fully understood. Our overarching objective is to utilize various strains of the model organism *C. elegans*, to investigate the relationship between WFS-derived PM and cognitive impairment. Ambient air PM2.5 exposure induces oxidative stress and activation of the unfolded protein response, suggesting disruption of protein homeostasis. However, adverse cellular stress responses in brain due to WFS-derived PM on the brain have not been fully investigated. To understand the cellular mechanisms of WFS exposure, we have utilized wild-type *C. elegans* and assessed different forms of stress outcomes. A synchronous population of wild-type animals were exposed to 1mg/ml WFS for 24 hours followed by staining with Dihydroethidium. Our data show that exposure to WFS increases reactive oxygen species and oxidative stress production. We also found that exposure to WFS tends to reduce the mitochondrial organelle content, measured through a cell permeable dye Mito-green that detects mitochondrial mass. Collectively, these preliminary findings indicate that cellular stress caused by exposure to WFS in *C. elegans* may be induced by mitochondrial dysfunction resulting from oxidative stress. To further test the effects of WFS exposure we plan to utilize a *C. elegans* strain called NeuroPAL that has stereotyped color map of all neuronal types, which will allow us to assess adverse WFS effects on neurons within nematodes.

Graduate Student/ERHS

5. Mechanisms of Piperonyl butoxide cytotoxicity and its enhancement with Imidacloprid and metals in Chinese hamster ovary cells.

Mai M. Awad, Piyawan Chailapakul, Mark. A. Brown, Takamitsu A. Kato

The widespread use of chemicals and the presence of chemical and metal residues in various foods, beverages, and other consumables have raised concerns about the potential for enhanced toxicity. This study assessed the cytotoxic effects of Piperonyl butoxide and its enhancement by combination with major contamination chemicals, including Imidacloprid and metals, using different cytotoxic and genotoxic assays in Chinese hamster ovary (CHO) cells. Piperonyl butoxide exhibited elevated cytotoxic effects in poly (ADP-ribose) polymerase (PARP) deficient CHO mutants but not in Glutathione S transferase deficient CHO mutants. PBO cytotoxicity was enhanced by PARP inhibitor, Olaparib. PBO cytotoxicity was enhanced with co-exposure of Imidacloprid, Lead Chloride, or Sodium Selenite. PBO induces γH2AX, and apoptosis. The induction of DNA damage markers was elevated with PARP deficiency and co-exposure to Imidacloprid, Lead Chloride, or Sodium Selenite. Moreover, PBO triggers to form etch pits on plastic surfaces. These results revealed novel mechanisms of PBO cytotoxicity associated with PARP and synergistic effects with other environmental pollutants. The toxicological mechanisms underlying exposure to various combinations at different concentrations, including concentrations below the permitted limit of intake or the level of concern, require further study.

Guest researcher/ ERHS

6. A weakening of the blood-brain barrier is identified in a naturally occurring brain aging guinea pig

Kevin E. Ayala, Amanda Latham, Kristin Weninger, Kelly Santangelo, Karyn Hamilton, and Julie Moreno

The primary risk factor for developing neurological diseases such as dementia, Alzheimer's Disease, and Parkinson's Disease is aging. The world's aging population is predicted to more than double in the next three decades. This increasing aging population raises concerns, as the treatment options available are inadequate and there is an incomplete understanding of aging neuropathology. Presently, the known neurological consequences of aging include the accumulation of misfolded proteins, neuroinflammation, and memory loss. A system that's role is important for the integrity and security of the brain, is the blood-brain barrier (BBB). The BBB regulates biomolecular and cellular trafficking into and out of the brain. Dysregulation of the BBB allows neurotoxic biomolecules and peripheral immune signals to enter the brain, activating glial cells, and resulting in neuroinflammation. In this study, we analyzed brain tissue for glial inflammation and aggregation of misfolded proteins from male Dunkin Hartley guinea pigs at 5 and 12 months of age. We detected an increase in microglial and astrocyte cell number, as well as the presence of hyperphosphorylated tau and amyloid β1-42 in the 12-month-old animals. We then asked if the BBB integrity is also altered in aged animals compared to young controls. To test this, we used immunofluorescence microscopy to analyze major constituents of the BBB, including claudin V, aquaporin-4, and ve-cadherin. We found that these proteins critical to BBB function are modulated in the 12-month aged guinea pigs compared to the 5-month animals. These results indicate that the BBB is a vital component for protecting the brain and that as aging occurs, the BBB is weakened. Since BBB dysregulation naturally occurs with age, future studies will focus on the effects of peripheral infections, which are also known to weaken the BBB, on the aging brain. This research was funded by NIH NIH/NCATS Colorado CTSA Grant Number UM1 TR004399. Student support was provided by NIH T32 IMSD grant T32GM144856.

Graduate Student/ERHS

7. Clustered DNA Double Strand Breaks for Cancer Treatment via Multi-gRNA CRISPR/Cas9 System

Gamze Badakul, Neelam Sharma, Jac Nickoloff

Radiation therapy is one of the most common cancer treatments. Ionizing radiation generates DNA double-strand breaks (DSBs), a lethal type of DNA damage. The dominant pathway for repairing DSBs is non-homologous end joining (NHEJ), but homologous recombination (HR) is also important. Unrepaired or misrepaired DSBs can lead to cell death. KU70/80 and DNA-PKcs are essential for NHEJ. Clustered DSBs (DSBs separated by <200 bp) generate small DNA fragments that fail to activate DNA-PKcs kinase, inhibiting NHEJ and shifting repair toward HR. Thus, clustered DSBs are poorly repaired and more cytotoxic than dispersed DSBs. High LET radiation has a higher ionization density and therefore induces more clustered DSBs than low LET, which explains why high LET radiation is more cytotoxic at a given dose. We hypothesize that mimicking high-LET-induced clustered DSBs using CRISPR/Cas9 targeted to cancer-specific DNA sequences (e.g., indel mutations) will effectively kill cancer cells but spare normal cells. These DSBs are not targeted to specific genes, nor is CRISPR/Cas9 used for gene editing. Instead, cancer cell killing is achieved by inducing poorly repaired DSBs anywhere in cancer cell DNA. This approach prevents oncogenic pathway-dependent resistance. Our data indicate that the cytotoxicity of clustered DSBs depends on the number of small DNA fragments induced and this finding significantly increases available targets as 2-DSB clusters can be induced at indels and flanking (wild-type) sites, generating clustered DSBs in cancer cells but efficiently repaired dispersed DSBs in normal cells. Data also indicates that a single DSB targeted to each amplicon of highly amplified regions is also cytotoxic, and we are exploring the effectiveness of combination clustered DSBs targeted to amplified regions. These proof-of-principal studies represent the first approach to targeted cancer therapy that is oncogenic pathway-independent.

Graduate Student/ ERHS

8. Evolution in action: SARS-CoV-2 within-host population expansion, diversification & evolution in tigers, lions and hyenas at the Denver Zoo

Laura Bashor, Emily Gallichotte, Katelyn Erbeck, Mark Stenglein, Michelle Galvan, Lara Croft, Katelyn Stache, Jimmy Johnson, Kristy Pabilonia, Sue VandeWoude

Although numerous animal species are known to be susceptible to SARS-CoV-2, very little is known about transmission dynamics and patterns of viral evolution in nonhuman hosts. RNA viruses are able to adapt quickly to a new host environment following a spillover event, and the emergence of potentially adaptive within-host SARS-CoV-2 variants has been observed in domestic and wild species. Zoo animals have been infected with SARS-CoV-2 through contact with humans during the pandemic, and transmission of SARS-CoV-2 back to humans (spillback) has been reported. The purpose of this study was to examine patterns of transmission and evidence of host-specific adaptation following an outbreak of SARS-CoV-2 in Amur tigers (*Panthera tigris altaica*), African lions (*Panthera leo*), and Spotted hyenas (*Crocuta crocuta*) at the Denver Zoo. Two tigers (both aged 6 years), eleven lions (aged 1-9 years) and two hyenas (both aged 22 years) tested positive by RT-PCR for SARS-CoV-2 between October and December 2021. Positive nasal swab samples were obtained from all 15 individuals for multiple timepoints during this two-month period, and full SARS-CoV-2 genomes were sequenced at a high depth of coverage. Sequence analysis indicated a single spillover event into animals of the SARS-CoV-2 lineage AY.20, which was circulating in low frequencies in the human population during this period. We observed significant increases in within-host SARS-CoV-2 genomic nucleotide diversity over time. Signatures of positive selection were most evident in the nucleocapsid (N) gene, but differed among the three species. Two candidate adaptive mutations in the N gene were identified in lions and hyenas. This study describes cross-species transmission of an emergent virus at the human-wildlife interface in a zoo environment, and highlights the resulting potential for species-specific adaptation. Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number T32AI162691. A MARC Scholar was funded by a grant from the National Institute of General Medical Sciences of the National Institutes of Health: T34GM140958 This research was supported by a CVMBS CRC Award and CSU VPR's 'Accelerating Innovations in Pandemic Disease' initiative, made possible through support from The Anschutz Foundation. Computational resources were supported by NIH/National Center for Advancing Translational Science Colorado Clinical and Translational Science Awards grant UL1 TR002535.

Graduate Student/MIP

9. Mercury exposure reveals prey selection in wolves from Denali National Park and Preserve

Elisa S. Behzadi, Bridget Borg, Layne Adams, Gretchen Roffler, Angela Gastaldi, Benjamin D. Barst

Wolves are a keystone species in Denali National Park and Preserve (DNPP). Their diet habits influence cascading effects on other species and the integrity of DNPP's ecosystems. Understanding wolf diet provides key insights into species interactions and resource use and is vital for resource managers. Although wolves are typically viewed as obligate predators of ungulates, emerging research indicates that they have broader diets than previously thought. Reliance on aquatic resources (e.g., fish and marine mammals) has implications for exposure to methylmercury, an organometal produced mainly in aquatic habitats. To identify dietary variation in wolf diets from DNPP, we analyzed wolf fur samples from about 19 DNPP wolf packs. We analyzed the bulk nitrogen and carbon stable isotope values and total mercury concentrations of 51 wolf fur samples. Methyl mercury analysis was performed on selected samples. Total mercury concentrations in wolf fur ranged from 60.53 to 2094.32 parts per billion and concentrations were generally higher in individual wolves from the west of the park, where there is greater access to streams with spawning anadromous Pacific salmon. This is the first research demonstrating that interior Alaska wolves accumulate mercury through the probable consumption of Pacific salmon. Student support provided by NIH T35OD015130.

DVM Student/ Other

10. Detection of gastrointestinal parasites in fecal samples from stray cats in Wyoming shelter

Elizabeth Berry, Kaci Shaw, Valeria Scorza, Krista Bratlien, Rachel Isdale, Michael R. Lappin

Gastrointestinal parasites are common in cats with the highest prevalence rates often being in shelters and other crowded environments. Results of repeated testing of shelter cats during a mandatory hold period are rarely reported. The objective of this study was to estimate the prevalence of gastrointestinal parasites cats on admission to a Wyoming animal shelter and 4-5 days later. On intake, all cats were administered pyrantel pamoate. A fecal score was assigned to all samples using a standardized scoring system, all fecal samples were tested by microscopic examination following centrifugal flotation and a *Giardia/Cryptosporidium* immunofluorescent assay, and *Giardia* positive feces were assessed in 4 different PCR assays to determine whether the assemblages were zoonotic or feline-specific. None of the cats had diarrhea although parasites were identified in the first sample of 21 of the 100 cats and included *Toxocara cati* (7 cats), *Giardia spp*. (5 cats), *Taenia spp*. (5 cats), *Cystoisospora felis* (4 cats), and *Cryptosporidium spp., Ancylostoma spp.,* or *Capillaria spp*. in one cat each. Co-parasitisms were detected in 3 (3%) of the cats. A second sample was available for 64 cats with *Taenia spp.* (6 cats), *Toxocara cati* (3 cats), *Giardia spp*. (2 cats), *Cryptosporidium spp*. (2 cats), *Cystoisospora felis* (1 cat), and *Capillaria spp*. (1 cat) being detected in some cats. Administration of pyrantel may have lessened some parasites but a new parasitism was detected in 9 of these 64 samples. The *Giardia spp*. positive sample with adequate DNA for sequencing to date was the feline specific assemblage F by both the bg and gdh genes. The results indicate that stray cats often carry a variety of gastrointestinal parasites while showing no clinical signs and performance of fecal diagnostic assays once does not have 100% sensitivity. This work was funded by Nestle Purina. Student support was provided by the Center for Companion Animal Studies.

DVM Student/CS

11. Comprehensive Characterization of the Ovariectomized Sheep Model of Osteoporosis: A Systemic, Histomorphometric, and Proteomic Study

Katie Bisazza, Brad B Nelson, Katie J Sikes, Lindsey Burton, Emily Van Zeeland, Lucas Nakamura, Russell Anthony, Laurie Goodrich, Kirk McGilvray, Jeremiah T Easley

Ovariectomized (OVX) sheep are commonly used in osteoporosis research because they are comparable to humans in both bone size and microarchitecture. However, the cellular pathways involved in bone turnover in ovine models of osteoporosis have not been fully elucidated. The objective of our study is to comprehensively characterize the disease progression of osteoporosis using a previously established OVX-corticosteroid sheep model. Sixteen intact, naïve, skeletally mature ewes were enrolled in the study, ten (N=10) of which were assigned to the osteoporosis (OP) group and underwent OVX surgery to remove both ovaries and were administered a high-dosage regimen of corticosteroids for four months following surgery. The remaining six (N=6) animals served as age-matched healthy controls (IACUC approval #2060). All animals underwent data collection procedures at five time points: baseline, 3, 6, 9, and 12-months. Clinical bone density of the lumbar spine was measured via dual-energy X-ray absorptiometry. Iliac crest biopsies were collected and bone microarchitectural parameters assessed by micro-computed tomography and histomorphometry. Additionally, bone biopsy samples were analyzed for global untargeted proteomics. Systemic health was assessed by analysis of blood hematology, serology, and body weights. OP group bone density and microarchitecture was significantly altered compared to control animals after 3-months. The OP group also displayed significant hematologic and serologic changes at 3 and 6-months compared to controls. Proteomic analysis revealed a total of 146 extracellular matrix proteins, with 11 differentially expressed proteins at 6-months in the OP group compared to baseline samples. Of note, ANXA3 and ANXA11 were upregulated and COL14A1 was downregulated. Proteomic analysis of non-ECM associated proteins is ongoing. These results help to further characterize novel aspects of our large animal model of osteoporosis. Understanding the cellular pathways associated with bone loss can aid in future cellular therapy research and lead to novel pathway targets for disease treatment in humans. Research funded by the Preclinical Surgical Research Laboratory (PSRL) at CSU.

Graduate Student/ CS

12. Monte Carlo Determination of Detection Efficiency for Portal Monitoring

Noah J. Blair, Alexander Brandl

At ports of entry into the country, at high security events such as political or athletic gatherings of crowds, and at high risk locations, portal monitors are used to detect the presence of ionizing radiation and ensure radiological/nuclear materials do not fall out of regulatory control or are utilized with malicious intent. This work uses computer models to determine the probability of photon radiation being detected in polyvinyl toluene, a plastic scintillator material, for a range of source energies, truck positions relative to the detector, and cargo materials. These results are used to develop a model for operators of portal monitors to predict the activity of a radioactive source given the measured count rate and integrated count measurements. Research funded by Nuclear Regulatory Commission Grant Number 31310021M0028.

Graduate Student/ERHS

13. A liquid suspension of simulated wildfire smoke particulate increases cytotoxicity in RAW264.7 cells

Sean Boland, Adam Schuller, Alexandra De Garay, Emma Smith, Luke Montrose, and Julie A. Moreno

Increased wildfire activity and resulting smoke is a major public health concern which necessitates the study and characterization of toxic responses. Epidemiologic data shows that wildfire smoke particulate matter (PM) negatively impacts the cardiopulmonary system and disrupts immune responses. Fine smoke particles can bypass defense mechanisms and enter the deep lung. Lung resident macrophage toxicity is linked to smoke exposure, but the mechanism remains unclear. In this study, we investigate the role of simulated wildfire smoke induced macrophage responses over time (6, 12, and 24 hours) and at varying doses (500, 250, and 100 ug/cm2). We utilized a Raw264.7 macrophage cell line exposed to PM extracted from gravimetric filters containing smoke generated by burning Douglas fir needles at a smoldering temperature. PM extracted from the filter was resuspended in phosphate buffered saline (PBS) at a concentration of 1 mg/ml. To determine the extent of wildfire smoke induced cytotoxicity of the Raw264.7 cells, we performed the cell viability assay (CellTiter Blue) and will further assess caspase 3 and annexin V staining. At 6 hours only the 500 ug/cm2 dosage showed a significant decrease in cell viability. However, at 12 hours there was significant dose dependent decrease in viable cells. Again at 24 hours only the 500 ug/cm2 dosage showed a significant decrease in cell viability. To more fully elucidate mechanistic pathways, we plan to assess inflammatory response (TNF-α and TGF-β), nuclear extraction and analyzed NF-kB translocation (WES), mitochondrial ROS (MitoSOX), and mitochondrial permeability (MitoTracker Red CMXROS). We also plan to extend the study to primary macrophage models to increase translatability.

Graduate Student/ ERHS

14. Sheep to Human: Unraveling the Translational Potential of Modic Changes in Disc Degeneration

Andres Bonilla, Brad Nelson, Jeremiah Easley

Modic changes (MCs), characterized by alterations in MRI signal intensity within vertebral bone marrow, have gained significance due to their association with Intervertebral Disc Degeneration (IDD) and subsequent low back pain. This study focused on categorizing MCs observed in mature sheep, a valuable large animal model for IDD research, to enhance our comprehension of MCs' variability in IDD progression. Three mature sheep underwent partial discectomy at the L2-L3, L3-L4, and L4-L5 levels to induce IDD, approved by the Colorado State University Animal Care and Use Committee. MRI scans were conducted pre-surgery and at 8, 16, and 30 weeks post-surgery, using T1W and T2W sequences in a sagittal view covering 36 endplates. An observer, blinded to the conditions, analyzed the images using Horos imaging software, and the data underwent variance analysis. The baseline assessment revealed type 2 MCs in only two endplates out of 36. At 8 weeks, four endplates exhibited type 2 MCs, increasing to eight at 16 weeks and peaking at 11 by 30 weeks. This progressive trend indicated a consistent rise in endplates demonstrating MCs over the study duration. Statistical differences in the number of MCs over time, especially from baseline to the study's conclusion, highlighted the dynamic nature of MCs in this sheep IDD model. The rapid emergence of type 2 MCs within a limited timeframe underscores the dynamic nature of MCs in this animal model. This study provides essential insights into the swift progression of Modic changes type 2, emphasizing the evolving nature of spinal degenerative alterations. Furthermore, the specific localization of these changes offers crucial guidance, informing targeted therapeutic strategies for optimal patient outcomes.

Graduate Student/CS

15.

Detection of a stat5b (N642H) mutation in feline t cell neoplasia with droplet-digital pcr analysis (ddPCR)

Sydney Bork, Robert Burnett, Kelly Hughes, Emily Rout, Cora Rutledge, Paula Schaffer, Janna A Yoshimoto, Craig Webb, Anne Avery

Lymphoma is the most frequently diagnosed neoplasm in cats and often manifests as small cell T cell intestinal lymphoma (SCL). The JAK/STAT pathway is commonly mutated in various T cell neoplasms and features Signal Transducer and Activator of Transcription 5b (STAT5B). Mutations in this transcription factor induces uncontrolled cellular proliferation, anti-apoptotic responses, and angiogenesis. A notable A to C transversion single-nucleotide polymorphism (SNP), resulting in an activating Asn to His mutation in the STAT5B (N642H) protein has been described in feline SCL cases. This prompted our laboratory to develop a droplet-digital PCR (ddPCR) assay to detect this mutation. Examining samples from three distinct cat cohorts—peripheral blood from young cats, intestinal biopsies from SCL-diagnosed cats, and cats with CD4 T cell leukemia was performed. In the young peripheral blood group (n=37), all cats were polyclonal for T cell Receptor (TCR) rearrangements by PARR, and ddPCR indicated an absent STAT5B mutation. The SCL group (n=27), confirmed by two board-certified pathologists, displayed clonal TCR rearrangements, with the STAT5B N642H mutation present in 18 cats (66.7%) and suspected in 3 (11.1%). In the CD4 leukemia group (n=44), all cats exhibited clonal TCR rearrangements, and the STAT5B N642H mutation was present in 22 cats (50%) and suspected in 3 (6.8%). This study demonstrated that cats diagnosed with SCL, and CD4 T cell leukemia frequently carry the STAT5B N642H mutation, shedding light on its prevalence and potential implications in the diagnosis and treatment of feline T cell neoplasia.

Resident/ MIP

16. Intrinsic and synaptic properties of posterior hypothalamus neurons in male and female rats

Courtney A. Bouchet, Kevin E. Ayala, Christopher E. Vaaga, Bret N. Smith, Brent Myers

Cardiovascular diseases, the leading cause of death globally, are exacerbated by stress; however, the neurobiological mechanisms linking stress to cardiovascular outcomes are not well understood. Neurons within the posterior hypothalamus (PH) are sensitive to stress and project to brain regions that regulate endocrine stress responding and cardiovascular physiology. The PH is critical for both neuroendocrine stress responding and maintaining balanced cardiovascular function, although investigations of the PH to this point have been limited to males. Additionally, physiological properties of these neurons have not been reported. Given that stress-cardiovascular comorbidities are more prevalent in females and preliminary data show that the PH is responsive to chronic variable stress in a sex-specific manner, understanding the physiological features and activity of the female and male PH is of the upmost importance. Therefore, this project aims to determine intrinsic and synaptic properties of neurons within the PH of male and female Long-Evans rats (n = 5 per sex, PND 102-136). Using whole-cell patch clamp electrophysiology, action potential firing at rest was used to determine the proportion of quiescent cells at rest, basal firing frequency, and action potential properties including half-width and phase-plane analysis. Intrinsic excitability was further evaluated by constructing a frequency-current (FI) curve to evaluate action potential dynamics evoked by current injection. Additionally, spontaneous excitatory and inhibitory synaptic input onto each neuron was evaluated in voltage clamp. Overall, the data presented indicate that the PH is composed of a heterogenous population of neurons that are not distinguishable between male and female; however, with stimulation PH neurons are more excitable in females compared to males. Given that Future experiments will investigate alterations in firing properties induced by chronic variable stress and could identify factors underlying cardiovascular susceptibility and resilience. This work was supported by NIH R01 150559 awarded to Dr. Brent Myers.

Post-doctoral Fellow/BMS

17. CD206 agonism stimulates growth of canine histiocytic sarcoma cell lines

Rachel V. Brady, Lisa J. Schlein, Travis K. Meuten, Kristen H. Farrell, Douglas H. Thamm

Histiocytic sarcoma (HS) is an aggressive cancer of macrophage or dendritic cell origin with a grave prognosis in both dogs and people. These tumors are heavily infiltrated by immunosuppressive tumor-associated macrophages (TAMs). Both TAMs and HS cells commonly express a scavenger receptor, CD206, which mediates phagocytosis and immune homeostasis. Treating TAMs with a CD206 agonist results in apoptosis or repolarization to an anti-inflammatory phenotype. The effect of CD206 agonism on HS cells remains unknown. Therefore, the aim of this study was the demonstrate the effect of a small molecule CD206 agonist (972) on canine HS cells. Three HS cell lines were incubated with increasing concentrations of 972. Viability was assessed through the IncuCyte NucLight cell labeling system and resazurin blue fluorescence. Changes in downstream signaling pathways were interrogated via a NF-κB luciferase reporter assay and western blots for phosphorylated ERK and AKT. In contrast to its impact on murine and human TAMs, CD206 agonism repeatably stimulated growth in all HS cell lines. CD206 agonism also upregulated NF-κB signaling in all cell lines, and upregulated phosphorylated ERK and AKT in two cell lines. These data indicate that canine HS cell growth is stimulated via agonism of CD206. NF-κB, ERK and AKT are all potential therapeutic targets of interest that may be routinely upregulated during HS disease. Work is ongoing to explore the impact of inhibiting these targets, validate on-target effects with CRISPR-Cas9, and to investigate the impact of CD206 agonism on primary canine macrophages. This research was funded by AKC Canine Health Foundation Clinician-Scientist Fellowship Program (award number 03033-E); Morris Animal Foundation (award number D22CA-500), and NIH Ruth L. Kirschstein Institutional NRSA Training Grant (award number T32OD010437).

Graduate Student/ CS

18. Determination of backyard chicken plasma protein reference data using protein electrophoresis

Jeffrey C. Brandon, A. Russell Moore

The existing literature concerning biochemical reference data in domestic chickens has predominantly concentrated on commercial flocks, overlooking backyard chickens. Recently biochemical reference intervals were established for non-commercial flocks in Colorado. However, the dye-binding method employed by biochemistry analyzers for measuring albumin cannot accurately determine albumin in low concentrations. To ensure veterinarians are equipped to serve the growing number of backyard flocks, this study aimed to develop population-based reference intervals for plasma protein biochemistry measurements in backyard chickens (*Gallus gallus domesticus*) using protein electrophoresis. Heparinized blood samples were collected between May and July of 2023 from 150 non-commercial adult chickens that were deemed healthy and not in molt from 7 different privately owned backyard flocks in Colorado. Heparinized plasma samples were analyzed on a Cobas c501 analyzer to determine total protein and a mammalian albumin kit using the bromocresol green method. The globulin fraction and A/G ratio were calculated. The samples were then analyzed using agarose gel protein electrophoresis to determine albumin, globulin fractions, and the A/G ratio. Reference intervals were generated using Microsoft Excel (Microsoft Office 2016; Microsoft) and the Reference Value Advisor Software, according to ASVCP guidelines for each method of analysis and the results were compared. Non-partitioned reference intervals for electrophoretic fractions were generated using non-parametric methods and were: Albumin=1.39-2.95 g/dL, Alpha 1=0.13-0.32 g/dL, Alpha 2=0.33-0.91 g/dL, Beta=0.55-2.79 g/dL, Beta 1=0.28-1.4 g/dL, Beta 2=0.33-1.80, Gamma=0.25-1.29 g/dL. This study established plasma protein electrophoresis reference intervals for backyard chickens in Colorado, which can be utilized in similar circumstances. This work was supported by the Center for Companion Animal Studies.

DVM Student/MIP

19. Immune modulating effects of feeding a synbiotic supplement to cats

Krista Bratlien, Lyndah Chow, Jennifer Hawley, Kara Maslyn, Steven Dow, Michael Lappin

Understanding the immunologic effects of probiotics in cats is important to possibly aid in the management of feline diseases, including viruses. The probiotic *Enterococcus faecium* strain SF68 has been shown to have immune modulating properties, including studies showing supplemented cats have less recurrent signs of feline herpesvirus-1 and increased CD4+ lymphocytes over time. We are now able to analyze the immune system of cats more completely by combining total RNA sequencing with select cytokine/chemokine production to assess specific cellular changes. *E. faecium* strain SF68 has been combined with the prebiotic, psyllium, which is commercially available as a synbiotic (Fortiflora SA, Purina PetCare). The primary objectives of this study were to further define the immune modulating effects of the synbiotic in healthy cats using total RNA sequencing (Novogene) of peripheral blood mononuclear cells (PBMC) and a feline cytokine/chemokine panel (Milliplex MAP). In addition, a feline infectious peritonitis virus in vitro inhibition test using the Fcwf-4 macrophage cell line was used to assess for antiviral activity in the serum of supplemented cats. Compared to controls, the cats fed the synbiotic had 45 up regulated genes and 33 down regulated genes in PBMC samples collected on Day 28. A KEGG pathway analysis was completed and revealed a significant up regulation in the TNF-alpha pathway and down regulation in the IFN-gamma and IFN-alpha pathways. The cytokine/chemokine analysis showed significant increases in IL-12 in supplemented cats. Serum from supplemented cats induced numerical decreases in FIPV mediated cytotoxicity. These results provide more information documenting that this probiotic is an immune modulator in cats. Research funded by Nestle Purina PetCare. Student support provided by the Center for Companion Animal Studies.

DVM Student/ CS

20. Collective storage of insect specimens increased false positive identification of viral infection

Ali L. Brehm, Mark D. Stenglein

Insect collection and storage for use in research can be conducted in a variety of ways. Largely, insects collected in the field are frozen or stored in a preservative like ethanol or RNAlater for use in downstream applications. At the time of collection, many individuals are stored via method of choice en masse. Previous studies have shown that for bacterial microbiome information, insects stored together do not benefit from surface sterilization to ensure that no external bacteria skew results. However, while conducting a prevalence over time study in Drosophila melanogaster for galbut virus, a multipartite dsDNA virus, we noticed that the parent generation, which were created using a fixed percent of infected individuals, were testing positive for galbut virus at a rate far higher than was input. To determine the source of the false positive results, we combined freshly dead flies from a galbut virus uninfected white-eyed fly line (W1118) with a red-eyed line (DGRP-517) that is persistently highly infected with galbut virus. After several days in the freezer, white-eyed flies were collected and screened for galbut virus infection with qPCR. In doing this, we found that despite being uninfected before entering the plastic bag with infected flies, the white-eyed flies were positive for galbut virus via qPCR after storage with infected flies. These results suggest that en masse storage of insects is not the best way to preserve specimens, especially for use in viral infection or prevalence studies. Research funded by NSF IOS 2048214.

Graduate Student/MIP

21. Development of canine CAR-T cells targeting the disialoganglioside GD2

Samuel Brill, Michael Yarnell, Lillie Leach, Isabel Brandtjen, Terry Fry, Douglas Thamm

Chimeric antigen receptor (CAR)-T cell therapy is a form of immunotherapy which has demonstrated remarkable efficacy in blood cancers, boasting up to 90% remission rates in some forms of leukemia/lymphoma. Despite success in blood cancers, CAR-T cell efficacy in solid tumors is lacking. There are many forces driving the immune-tumor microenvironment that lead to CAR-T cell dysfunction in the context of solid tumors. While mouse models have been integral to CAR-T cell development to date, modeling the complex tumor-immune dynamics scales poorly in mice. Dogs provide a natural animal model to evaluate cell-based immunotherapy owing to their similar immune systems, natural history of cancer, and clinical care. We have developed a system for generating canine CAR-T cells as a first step to develop a canine model for CAR-T cell therapy. We demonstrate that the tumor associated antigen, disialoganglioside GD2, is expressed on canine osteosarcoma and melanoma cell lines. We have optimized culture conditions for T cell proliferation and activation, and demonstrated efficient CAR transduction using a gamma-retroviral vector. Finally, we demonstrate that primary canine T cells transduced with a GD2-targeting CAR can specifically kill GD2+ tumor, while sparing GD2- cells. Together, these data establish a platform for designing and evaluating canine derived CAR-T cells, demonstrate that GD2 is a targetable antigen on canine osteosarcoma and melanoma, and that canine CAR-T cells can specifically target GD2+ tumor cells. Student support provided by NIH F31CA265165.

DVM/PhD Student/ CS

22. Validation of a novel preclinical murine model of ankle overuse via mechanical induction

Hailey Brown, Emily Van Zeeland, Adam Chicco, Kelly Santangelo, Katie Sikes

Existing preclinical models of ankle injuries are either chemically- or surgically-induced and fall short of accurately mimicking the typical onset of injury and disease pathogenesis seen in the clinical setting. For this reason, this study aims to validate a novel preclinical model of chronic tendon, cartilage, and muscle overuse diseases in the ankle joint of mice. A total of 80 male and female wild-type mice will undergo one round of loading (30 minutes), in a custom system, at one of four severity levels: slow walking (SW), fast walking (FW), slow jogging (SJ), and fast jogging (FJ). Voluntary activity monitoring (Anymaze) and compulsory static weight bearing (Bioseb) will be used to evaluate mobility prior to injury and on days 3, 7, and 14 post-injury. Limbs collected from the mice will then be processed for histology and graded for generated overuse pathology, focusing on the Achilles tendon, gastrocnemius muscle, and the tibiotarsal joints. All data will be analyzed in GraphPad Prism using a two-way ANOVA for limb, time, and limb/time interaction factors. Preliminary static weight-bearing data for the slow walking (n=10) cohort of mice shows a significant change in weight bearing of the two hindlimbs at days 3 and 7 when compared to baseline measurements, with the mice favoring the uninjured limb. At day 14, there is no longer a significant difference in weight bearing when compared to baseline measurements. This data suggests that one round of loading successfully affects how animals are voluntarily loading their limbs. However, measurements from day 14 demonstrate a possible recovery from the initial injury period and indicate that either further rounds of loading at this low severity level, or a higher severity level may be needed to induce a chronic injury state. Research funded by a CVMBS CRC Award.

Undergraduate Student/CS

23. Range of motion between adjacent cervical vertebrae in horses with cervical vertebral compressive myelopathy

Dylan Burton and Dr. Yvette Nout-Lomas

Cervical vertebral compressive myelopathy (CVCM) occurs when portions of the cervical spinal canal become stenotic, resulting in spinal cord compression. CVCM is the most common noninfectious neurologic disease in horses, and ultimately leads to unusable horses which directly impacts the equine industry. Our aim is to evaluate if range of cervical vertebrae joint movement is different between CVCM and control, unaffected, horses. We hypothesize that horses with CVCM have increased range of motion which contributes to vertebral instability. Medical records and imaging reports of 19 CVCM and 10 control horses between 2015 and 2023 were reviewed. Measurements of joint angles between adjacent vertebral bodies on survey radiographs and neutral, flexed, and extended myelographic radiographs were determined. Evaluation of survey radiographs showed no differences in joint angles between the two groups. 6 CVCM horses were compressed at 1 site, 8 at 2 sites and 5 at 3 sites. In CVCM horses, 9 were compressed at C3-4, 8 at C4-5, 9 at C5-6, 9 at C6-7, and 2 at C7-T1. The average range of motion between adjacent cervical vertebral bodies based on myelography for controls and CVCM cases, respectively, are: C3-4: 24°vs28°; C4-5: 28°vs31°; C5-6: 27°vs28°; C6-7, 27°vs30°. Evaluation of controls compared to compressed sites only shows range of motion at C3-4: 24°vs31° (n= 8); C4-5: 28°vs33° (n=8); C5-6: 27°vs26° (n=8); C6-7, 27°vs28° (n=6). In reviewing this preliminary data, we conclude there does not appear to be a substantial difference in range of motion of cervical joints between control and CVCM horses. This data contributes to our further understanding of the relevance of biomechanics of the neck in the pathogenesis of CVCM. Research supported by Dr. Yvette Nout-Lomas, Equine Neurology fund. Student support provided by USDA-NIFA-AHDR-COLV-23.

DVM Student/ CS

24. Radiation Therapy as a Treatment for Urinary Obstruction Secondary to Urothelial Carcinoma in Dogs

Madelyn Burtz, Mary-Keara Boss, Susan M. LaRue, Tiffany Martin

Transitional Cell Carcinoma (TCC) is the most common urinary tumor in dogs, often invading the bladder wall, trigone, urethra, and prostate and can lead to obstruction of the outflow of urine at the level of the ureters or urethra. Urinary obstruction indicates advanced disease and is considered a medical emergency. Radiotherapy (RT) with or without adjunct chemotherapy and non-steroidal anti-inflammatory drugs is often used for treating acute obstructions as well as loco-regional control of these tumors, but ideal treatment of urinary obstruction is still being investigated. The objective of this study was to evaluate the efficacy of multiple RT protocols in relieving symptoms of urinary obstruction secondary to tumor growth. Data was collected retrospectively from records from dogs with BRAF testing, biopsy, or cytology confirmed TCC, that showed evidence of either partial or complete urinary obstruction that were treated with RT at Colorado State University. Percent of patients experiencing relief of obstruction and time it took to experience relief of symptoms associated with obstruction was recorded. Number of dogs that required surgical intervention was recorded. Overall survival time and progression free survival time were analyzed in comparison to location of outflow obstruction and radiotherapy protocol. Various RT protocols included 2.7-8 (median 5.4) Gy per fraction for a total of 6-57 (median 34) Gy in 1-20 (median 6) fractions. Out of 38 patients included, 29 patients unobstructed from RT (66%), the remaining were either euthanized prior to completion of treatment or had surgical intervention. RT may be a successful salvage option for dogs presenting with urinary obstruction caused by urothelial carcinoma. Student support provided by Boehringer Ingelheim Veterinary Scholar Program.

DVM Student/CS

25. Testing for Chronic Wasting Disease in Elk on the Western Slope of RMNP by using PMCA and RT-QUIC

Jessi Campbell, Kirsten Marshall, Kendall O'Brien, Analeis Cofield, Mark Zabel

Chronic Wasting Disease (CWD) is a transmissible prion disease that causes spongiform encephalopathy in cervids such as elk, deer, and moose. This study looks at the transmission of CWD in Elk on the Western Slope of Rocky Mountain National Park (RMNP), which has not been reviewed. Elk have previously tested positive on the Eastern Slope, and any positive samples on the Western Slope indicate the spread of CWD, continuing the surveillance of the disease. It is suspected that CWD is throughout RMNP and due to the migration patterns of elk, has spread to the Western Slope. Based on previous studies, CWD has spread from Colorado (discovered in the 1960's) to over 30 states and 6 Canadian provinces. Western Blotted samples from Protein Misfolding Cyclic Amplification (PMCA) and Real-Time Quaking-Induced Conversion (RT-QUIC) were utilized to test fecal, water, and plant samples on the Western Slope of RMNP in Onahu Creek. For both the Western Blotted samples and RT-QUIC, the data showed inconclusive results. The importance of this study includes disease surveillance with faster diagnostic testing. Through these measures, this study aims to help control this disease via monitoring trends while a cure is under development. Student support provided by USDA-NIFA-AHDR-COLV-23.

DVM Student/ MIP

26. Gut-Brain-Axis influences Manganese-Induced Neurobehavioral Abnormalities and inflammation in the enteric nervous system

Celine Campos, Sydney Risen, Grace Weismann, Mila Trombley, Peyton Shirley, Molly Schmanke, Karin Streifel, and Julie Moreno

Manganese (Mn) is an essential trace metal, however in excess it is a potent neurotoxin. Epidemiological studies have shown that children are particularly vulnerable to Mn exposure and cognitive and behavioral deficits can occur to this heavy metal exposure. Interestingly, the first site Mn accumulation is the enteric nervous system (ENS) via the gastrointestinal tract (GI). The ENS is composed of glial and neuronal cells that may be affected similar to the same cells within the CNS . We hypothesize that there will be a correlation between behavioral and cognitive deficits relative to enteric glial inflammation prior to CNS inflammation. To test this, mice were administered environmental-relevant levels of Mn, 50 mg/kg MnCl2, daily via drinking water from postnatal days 21-90. Throughout the study we evaluated locomotor function, memory and learning, and glial activation in both the CNS and ENS weekly. Preliminary results from these studies in weeks 10 through 12 have replicated sex-dependent behavioral changes in the juvenile mice previously observed. Initial analysis of the glial number (anti-GFAP) and activation (anti-S100□□, show an increase in GFAP and S100□ positive cells in the ENS prior to the CNS. These data indicate that exposure to Mn during development leads to inflammatory activation of the cells in the ENS prior to CNS. These findings lead us to question if Mn induces a neurotoxic effect on the gut-brain-axis leading to the behavioral modifications that mimic attention deficit hyperactivity disorder (ADHD) or other neurodevelopmental disorders. Funding for this research came from the Society of Toxicology, Regis University Faculty Development and University Research and Scholarship Grants, CSU REU/NSF student funding, Supported by the Colorado State University Grant Number NIH PREP R25GM148297, and the Department of Environmental and Radiological Health at Colorado State University for use of equipment and supplies.

PostBacc/ERHS

27. Innate fear responses to distal and proximal predator threats

Jordan N. Carroll, Brent Myers, Indira M Raman, Christopher E. Vaaga

To survive predation, animals must be able to detect and appropriately respond to predator threats in their environment. Such defensive behaviors are innate: suggesting dedicated neural circuits for the detection, sensorimotor integration, and execution of appropriate behaviors. Ethologically, distal threats (i.e., sweeping visual stimuli) trigger 'passive' avoidance strategies, such as immobility to avoid predator detection. Conversely, more proximal threats (i.e., looming visual stimuli) trigger 'active' avoidance strategies, such as fleeing. The goal of the present study is to examine innate fear responses when the behavioral strategy is determined by extrinsic factors. Specifically, we investigate how environmental and physiological conditions modulate innate immobility responses in mice, with a particular emphasis on understanding the conditions that alter behavioral responses to repeated stimuli. We demonstrate that in both male and female mice looming and sweeping visual stimuli elicit immobility in the absence of a nest to flee towards. However, looming stimuli triggered comparatively prolonged immobility bouts. Furthermore, when looming visual stimuli were repeated at intervals as short as 5 minutes, immobility responses decreased across trials in response to both looming and sweeping stimuli, suggestive of fear suppression. To begin testing whether the fear suppression represents safety learning, we tested animals at intervals of 1 hour and 24 hours. Finally, we investigated how acute stress may impact innate fear responses by randomly applying foot shocks to the mice 24 hours prior to the presentation of a looming visual threat. Together, our results indicate that when mice engage in passive avoidance strategies triggered by distal or proximal threats, the vigor of the response scales with threat proximity. Further, our results demonstrate that freezing responses are modified by repeated threat exposure as well as stress-inducing stimuli. Research supported by NIH award R00NS119783.

Graduate Student/ BMS

28. Effects of acute stress on periaqueductal gray spontaneous synaptic currents

Ana Valeria Castro Romero, Kevin E. Ayala, Christopher E. Vaaga

The midbrain periaqueductal gray (PAG) is heavily implicated in fear behaviors and is highly interconnected with different regions of the extended limbic system. The PAG is involved in risk assessment and behavioral responses to threat, and in fact, activation of the PAG drives fear responses in both humans and animal models. However, how the PAG integrates both intrinsic and extrinsic synaptic inputs is not entirely understood. Furthermore, whether and how acute stress impacts synaptic transmission in the PAG, and the resulting effects on fear behaviors, is poorly understood, despite clinical and pre-clinical evidence that stress, including PTSD, alters fear behavior. The goal of the present study was to examine how acute stress alters spontaneous excitatory and inhibitory synaptic transmission in the ventrolateral PAG (vlPAG), which selectively drives freezing behaviors. Our results indicate that acute stress alters the relative strength and frequency of both excitatory and inhibitory synaptic currents in the vlPAG in a sex-dependent manner. Our preliminary data indicate that females are more susceptible to changes in synaptic transmission following acute stress. Together these results motivate further testing the hypothesis that acute stress alters synaptic transmission within the vlPAG, and examining how such changes alter fear behavior. Research supported by NIH award R00NS119783. Student support provided by MCIN.

Graduate Student/BMS

29. Molecular prevalence of select vector-borne pathogens in Thai client-owned anemic dogs in Bangkok and Nakhon Si Thammarat

Nida Chornarm, Sukullaya Ritthikulprasert, Mathurot Suwanruengsri, Melissa Brewer, Michael R. Lappin

Vector-borne pathogens are common causes of anemia in dogs and can be fatal. The aim of this study is to use molecular techniques, blood smear, and serological assays to investigate the prevalence of select vector-borne pathogens in anemic client-owned dogs presented to 2 veterinary teaching hospitals in Thailand. Frozen ethylenediaminetetraacetic acid (EDTA) anticoagulated whole blood was collected from a total of 217 dogs with anemia (hematocrit ≤ 35%) from the Bangkok area (199 dogs) and dogs in the Nakhon Si Thammarat area (18 dogs). The mean hematocrit was 29% (range 13.5-35%). A quantitative polymerase chain reaction (qPCR) assay was used to amplify the DNA of *Babesia spp.*. Conventional PCR assays were used to amplify the DNA of Babesia spp., Hemoplasmas, *Bartonella spp*., *Ehrlichia spp., Anaplasma spp., Neorickettsia spp., Wolbachia spp*., and *Rickettsia spp.*. Microscopic examination of thin blood smears was performed in 98 dogs. A commercially available kit (SNAP4DxPlus; IDEXX) to detect *Dirofilaria immitis* antigen and antibodies against *Anaplasma spp., Borrelia burgdorferi*, and *Ehrlichia spp*. was used to assay 109 dogs. DNA of at least one organism was amplified from 29 of the 217 dogs (13.4%) and included DNA of *Ehrlichia spp*. (19 dogs; 8.8%), *Rickettsia felis* (5 dogs; 2.3%), *Mycoplasma spp*. (4 dogs; 1.8%), *Anaplasma spp*., (3 dogs; 1.4%), or *Wolbachia spp*. (3 dogs; 1.4%). The results of a thin blood smear were positive in 3 dogs (*Ehrlichia canis*, *Hepatozoon canis*, and *D. immitis* microfilaria). Antibodies against *Anaplasma spp.*(12 dogs; 11%), *Ehrlichia spp*. (48 dogs; 44%), or *D. immitis* antigen (4 dogs; 3.7%) were detected in 53 of 109 dogs (48.6%). Discordant results were detected amongst the different tests for some dogs. The results support that canine vector-borne diseases are common in these two areas of study and molecular techniques should be used in combination with other diagnostic methods. Research and student support provided by the Center for Companion Animal Studies.

Graduate Student/ CS

30. Effects of trace metals found in asphalt on plutonium uptake on extraction chromatography resins

Raissa Chunko, Ralf Sudowe

In case of a nuclear incident, standard radioanalytical techniques must be available to analyze radionuclides in unusual matrices. Radiochemical analysis of samples in standard matrices of soil, water, and air are very well established; however, much less research has been conducted on the effect of unusual matrices such as steel, concrete, glass, and asphalt. In the event of a detonation of an improvised nuclear device (IND) in an urban environment, the standard separation techniques used for the determination of plutonium in asphalt samples originating from roadways and roofing shingles must be rigorously tested to provide useful insight on the characteristics of the special nuclear material. Batch studies were used to determine the changes in uptake of plutonium on commercially available extraction chromatography resins in the presence of trace metal components found in asphalt including aluminum, iron, and manganese at possible concentrations found in asphalt samples. In these studies, cations with a +3-oxidation state exhibited a positive effect on the uptake of plutonium on the extraction chromatography resins. Aluminum increased the sorption of plutonium only on DGA and TRU resins especially at increasing concentrations. Iron very unexpectedly increased the sorption of plutonium on all resins particularly at high concentrations. From this data, it can be assumed that the contaminates found in asphalt with a +3-oxidation state may act as salting out agents and increase the sorption of plutonium on the extraction chromatography resins and their presence will need to be considered during the development of a rapid separation technique for plutonium from asphalt samples. Research funded by the Mountain and Plains Education Research Center.

Graduate Student/ERHS

31. Implementation of the Ottawa Morbidity and Mortality Model in a veterinary teaching hospital

Cassidy M. Coats, Kristin M. Zersen, Catriona MacPhail, Petra Cerna, Tara Marmulak, Kelly Hall

To determine the effect on the perceived quality of morbidity and mortality (M&M) rounds in a veterinary teaching hospital, the Ottawa Morbidity and Mortality Model (OM3) was instituted in the Fall of 2021. A survey was emailed to participants at the beginning of the study period (Fall 2021, "PRE") and at the end of the academic year in 2022 (POST-Y1) and 2023 (POST-Y2). To evaluate individual monthly M&M round sessions, a separate survey was emailed to participants after each session during the first year. From PRE to POST-Y1 (PRE to POST-Y2) findings included: 1) a 19% (22%) increase in "agree" responses when asked if M&M rounds had a significant impact on the quality of care they provide on a section/service level; 2) a 10% (27%) decrease in "agree" responses when asked if M&M rounds needed improvement; 3) a 7% (9%) increase in "agree" responses when asked if M&M rounds can lead to better outcomes for patients. There was an approximately 25% increase in the average percentage of M&M rounds thought to effectively address cognitive issues between PRE and POST-Y1, and a 13% increase between PRE and POST-Y2. There was an approximately 25% increase in the average percentage of M&M rounds thought to effectively address systemic issues between PRE and POST-Y1, and a 20% increase between PRE and POST-Y2. The OM3 model improves recognition and understanding of both cognitive biases and systemic issues in the hospital and is an effective model for M&M rounds in a veterinary teaching hospital setting.

Intern/ CS

32. Determining therapeutic targets for peripheral t cell lymphoma, not otherwise specified in canines

Sara Cook, Eileen Owens, Janna Yoshimoto, Anne Avery

Human peripheral T Cell Lymphoma not otherwise specified (PTCL-NOS) is a proposed subset of T cell lymphoma. PTCL-NOS is a rare neoplasm in people but is relatively common in dogs. We hypothesize that the dog can be a useful pre-clinical model to study human PTCL. The goals of this study were to, 1) identify molecular pathways that are shared with human PTCL that could be targeted by drug therapy, and 2) Develop an in vitro system for evaluating the effect of these drugs on primary PTCL cultures. Using RNA sequencing data from 33 dogs, we found that the PI3K-AKT pathway is upregulated in canine PTCL, similar to findings in the human counterpart. We then developed two methods for determining the ability of drugs to inhibit this pathway and cause cell death. First, we demonstrated that constitutively phosphorylated AKT could be detected in primary PTCL cells using intracellular flow cytometry with an antibody specific for pAKT. This assay was then used to demonstrate inhibition of AKT phosphorylation following treatment with a PI3K inhibitor. Second, we optimized an LDH cytotoxicity assay, determining the number of cells and culture conditions best suited to evaluate cell death in the presence of PI3K inhibitors. Together, these results further confirm activation of the PI3K-AKT pathway in PTCL-NOS cells and provide a potential target for therapeutics. Additionally, this work confirms methods to be used in the future to determine potential efficacy of other therapeutics. Research funded by the Clinical Hematopathology laboratory and the Center for Companion Animal Studies Young Investigator Grant. Student support provided by NIH T32GM135528.

DVM/PhD Student/MIP

33. Tolerability of long-term cannabidiol supplementation to healthy adult dogs

Isabella Corsato Alvarenga, Kim Wilson, and Stephanie McGrath

Cannabidiol (CBD) has therapeutic potential in companion animals. Shorter-term studies have determined that CBD is well tolerated in dogs with mild adverse effects and an increase in alkaline phosphatase (ALP) activity. There is need to assess CBD's long-term tolerability. The goal of this study was to determine the long-term tolerability of CBD administered PO to healthy dogs for 36 weeks at dosages of 5 and 10 mg/kg body weight (BW)/day. Our hypothesis was that CBD would be well tolerated by dogs. Eighteen healthy adult beagle dogs were randomly assigned to 3 groups of 6 each that received 0, 5, or 10 mg/kg BW/day CBD PO. Dogs were adapted to their housing for 3 weeks and received treatment for 36 weeks once daily with food. Adverse events (AEs) were recorded daily. Blood biochemistry profiles were monitored every 4 weeks. Data were analyzed as repeated measures over time using a mixed model, with significance at $\alpha = 0.05$. The 0 and 5 mg/kg treatment groups had similar fecal scores, and the 10 mg/kg treatment group had higher frequency of soft feces. No other significant AEs were noted. An increase ($P < .0001$) in ALP activity occurred in groups that received CBD, with a tendency to be higher in dogs receiving the highest dose. Remaining blood variables were within reference range. Chronic administration of CBD in healthy dogs at 5 mg/kg was better tolerated than 10 mg/kg. Although our data does not indicate hepatic damage, it is recommended to monitor liver function in dogs receiving CBD chronically. Research funded by Hill's Pet Nutrition.

Post-doctoral Fellow/ CS

34. Purification of Ra-226 legacy waste for production of Ac-225: from nuclear waste to cancer treatment.

Maelle Coupannec, Ralf Sudowe

Currently, Ac-225 is produced in limited quantities through radiochemical separation from Th-229 sources. The growing demand for Ac-225 in cancer therapy necessitates increased production. An alternative method involves irradiating Ra-226 targets with protons, neutrons, or gamma-rays. Radium targets can be obtained through the purification of byproducts from uranium mining or repurposing legacy needles from brachytherapy diverted from a radioactive waste landfill. A significant challenge in radium target production is achieving the high purity necessary for sustainable yield. Radium's chemical analogues, such as barium and strontium, may pose interference after recovering Ra-226 from waste material, making their separation crucial. Several proprietary extraction chromatographic resins developed by TrisKem Int. (Bruz, France) were studied to quantify and improve the separation of radium from its chemical analogues. The TK101 resin showed the highest distribution ratio and a Ra/Ba separation factor of 6 at 0.05M HNO_3. Subsequent studies were conducted to determine the optimal separation contact time, temperature, and resin adsorption capacity. Dynamic column studies revealed successful analogue separation, with 87±2.8% of Ra recovered in 2M HNO_3 with <1% Ba breakthrough and 94±1.8% of Ba recovered in 8M HNO_3, while Sr remained retained on the resin.

Graduate Student/ERHS

35. Evaluating jak/stat inhibitors as therapeutics for feline small cell intestinal epitheliotropic t-cell lymphoma

Shayla Curry, Sydney Bork, Emily Rout, Tatianna Travieso, Janna Yoshimoto, Anne Avery

Feline small-cell intestinal epitheliotropic T-cell lymphoma (SCL) is the most common subtype of malignancy in cats. Despite its prevalence, diagnosing and treating this disease remains challenging as its pathogenesis is unclear. SCL is believed to arise from chronic intestinal inflammation, making it difficult to differentiate lymphoma from pre-existing inflammation using histology. We propose that with progression from inflammation to lymphoma, gene expression associated with oncogenic pathways is upregulated. Our preliminary data indicates a proposed activating mutation, STAT5BN642H, is present in the clonal T-cell population in our feline lymphoma samples. STAT5B is essential to the T-cell signaling pathway, resulting in constitutive T-cell activation. This mutation presents a new therapy opportunity as current treatment options are limited to chemotherapy and glucocorticoids, which have several drawbacks. Our goal is to determine if the inhibition of the JAK/STAT pathway with Apoquel (oclacitinib), a JAK 1 and 3 inhibitor, can decrease feline T-cell proliferation and proinflammatory cytokines. We have established an in vitro culture system to measure T-cell proliferation and cytokine production in feline peripheral blood from cats. Apoquel shows preliminary promise in decreasing proinflammatory cytokines and proliferative markers in neoplastic feline T cells. This model could be translated to human GI lymphoma which resembles feline SCL in clinical signs, gastrointestinal location, histology, immunophenotype, and T-cell driver mutation. Research funded by the Center for Companion Animals Young Invesigator Award and Clinical Hematopathology Laboratory. Student support provided by NIH T32GM135528.

DVM/PhD Student/ MIP

36. Comparability of echocardiographic estimates of stroke volume in healthy dogs

Kate Davis, Lance Visser

Stoke volume is an important measure of cardiac function that can be noninvasively measured with 2D and pulsed wave echocardiography at different anatomic sites of the heart. Estimations of stroke volume from different sites should be equal in dogs without cardiac disease, but may differ because some are more challenging to acquire and measure compared to others. We hypothesize that there will be consistent bias in some of the methods of estimating stoke volume from 2D and pulsed wave echocardiography relative to others. Following a physical exam and screening questions, an echocardiogram with a series of standardized measurements was performed on ninety-two healthy dogs of various ages, weights, and breeds. Data showed that stroke volume measured at the aortic valve was significantly higher than stroke volume measured at the pulmonary valve (P= 0.03) and left ventricle (P=0.008). Results from this study will generate reference values to be used in clinical settings. Student funding for this research was generously provided by Boehringer Ingelheim.

DVM Student/CS

37. Comparison of a point-of-care assay and a laboratory analyzer for cardiac troponin (cTnI) in guinea pigs (Cavia porcellus)

Amanda L. Day, Kelly S. Santangelo, Ben Singh, Sarah M. Ozawa, João Brandão, Miranda J. Sadar

Cardiac troponin I (cTnI), a cardiac specific biomarker that is released into circulation following cardiac myocyte damage, is used as a non-invasive method for diagnosing heart disease in small animals. Increases following suspected myocardial injury in black-tailed prairie dogs and rabbits have been reported, although a commercially available assay has not been validated for most non-traditional species. The immediate goal of this study was to compare a cTnI ELISA from a reference laboratory to a point-of-care cTnI assay (i-STAT) in clinically healthy, subadult and adult guinea pigs of both sexes; longer-term aims are to establish reference intervals for both assays. Twenty guinea pigs were used. Six male, and six female, subadult guinea pigs were administered 20 mg/kg ketamine and 2 mg/kg xylazine intramuscularly for venipuncture, and collection was repeated on four males and six females, awake, seven days later. Eight adult males were anesthetized using isoflurane for blood collection. Samples were measured in duplicate using the i-STAT for all groups and analyzed by ELISA from the isoflurane group. Results from the point-of-care assay showed an overall median of 0.006 ng/mL (range 0-0.06 ng/mL) for all groups, with medians of 0.008 ng/mL (range 0-0.06 ng/mL) for ketamine/xylazine (n=12), 0.019 ng/mL (0-0.05 ng/mL) for awake (n=10), and 0.007 ng/mL (0-0.06 ng/mL) for isoflurane (n=8) groups. Results from the reference laboratory ELISA are pending. Heart disease is a relatively common cause of morbidity and mortality in guinea pigs, and this information may be valuable for clinical assessment of myocardial disease in this species.

Intern/ CS

38. Neutron flux in a howitzer drum and construction of a water moderated neutron irradiator

Anilu Diaz, Ralf Sudowe

The Department of Environmental and Radiological Health Sciences at Colorado State University is utilizing a variety of irradiators to study the effects of ionizing on materials and tissue. Two of the sources are neutron irradiators based on 1 Ci and 5 Ci plutonium/beryllium (PuBe) sources, respectively. Neutron activation analysis is utilized to measure the neutron fluence at various positions in a Neutron Howitzer containing the 5 Ci source and a water tank containing the 1 Ci source. By determining the neutron flux in both systems, neutron irradiation at different intensities will become available for future research at Colorado State University. Additionally, both the drum and tank will be excellent teaching tools as they demonstrate neutron moderation, neutron shielding, material activation, and fluence measuring. Manufactured by the Nuclear-Chicago Corporation, the Model NH-3 Neutron Howitzer Drum is constructed in such a fashion that the PuBe neutron source can be moved in and out of irradiation position. In the irradiation position, two samples may be exposed to neutrons from the source by placing them in one of two horizontal ports in the drum. Both drum and ports are shielded with paraffin, which allows moderation of the neutron flux to thermal energies. In the experimental study, multiple metal foils were activated in the drum by irradiating them up to the point of measurable activity. Using a High Purity Germanium (HPGe) detector, the activity of the foils is quantified. The results of the measurements were used to calculate the neutron fluence using known neutron capture cross sections. The calculated neutron fluence was then compared to the neutron fluence determined through a computational model of the drum using the Monte Carlo N-Particle transport code (MCNP). Using the principles and methods practiced on the Howitzer drum, a water moderated neutron tank was constructed as a secondary neutron irradiator. The compared experimental and modeled neutron fluence spectrum in the drum and tank are reported in support of future research efforts.

Graduate Student/ERHS

39. Farmer's perceptions and practices of antimicrobial use and resistance among front range colorado dairy farm

Solange Dubreuil, Sangeeta Rao, Kelly Still-Brooks

Antimicrobial stewardship establishes a baseline from which we can improve upon antimicrobial usage so we may optimize treatment outcome, prevent harm in patients caused by their overuse, and combat resistance. A survey of perceptions and practices of dairy farm owners, managers, and workers on antimicrobial use and resistance, and in-feed antimicrobial use through the Veterinary Feed Directive (VFD) was built with the purpose of reveal gaps in the understanding of antimicrobial stewardship. This will allow researchers to provide evidence-based recommendations to improve overall understanding of VFD regulations and judicious use of antimicrobials on dairy operations, as well as solidify VFD regulations for employee training. A comprehensive survey was built with varying degrees of complexity and formats. Qualtrics® was used to build the survey and the link was shared with the participants to collect and analyze the responses. The survey consisted of 72 questions for managers and producers, and 51 questions for workers. The survey was available in Spanish and accessible by link or QR code. Producers were contacted by phone, email, or in-person visits to their farms. The project was explained; different survey formats were shared with those interested in participating. Some of the surveys were responded remotely and some others were obtained in-person by scheduled visits to the farms. Among the 15 completed surveys so far, we found that 80 percent of participants have heard about "antimicrobial resistance" and also 80 percent of participants think that there should be more initiatives to promote responsible use of antimicrobials in the dairy industry. Some of the challenges presented were engagement and participation due to the survey's length and the lack of time and hesitance to approach antimicrobial subject due to COVID 19.

DVM Student/ CS

40. Generating physiologically relevant extracellular vesicles from bovine oviductal organoids

Brandi Dunn, Nico G. Menjivar, Ahmed Gad, Riley E. Thompson, Melinda A. Meyers, Fiona K. Hollinshead, Dawit Tesfaye

Many cattle production systems are now focused on improving reproductive efficiencies through implementation of assisted reproductive technologies, such as in vitro production (IVP) of embryos. Despite the increasing use of IVP, there remains an imperative need to optimize embryo culture conditions since current IVP practices yield inferior quality blastocysts quality and lower pregnancy rates compared to in vivo. We hypothesize that the lack of maternal oviductal factors in the IVP culture system leads to suboptimal conditions for embryo development. The primary aim of this project is to address the necessity for improved culture conditions through the integration of oviductal-like factors into the embryo culture media. A 3D model through the culture of oviductal epithelial cells, referred to as organoids, will be developed. These organoids will be subjected to treatment with steroid hormones, estradiol and progesterone, to mimic the in vivo environment of an oviduct under diestrus conditions. We believe that by stimulating the oviductal organoids models in vitro with the steroid hormones, it is possible to generate physiologically relevant extracellular vesicles (EVs) and these EVs can be potentially supplemented in IVP embryo culture media to enhance the yield and quality of blastocysts. For this, blastocysts from the different treatment groups will be assessed for cell count, mitochondrial activity, and gene expression. Student support provided by USDA-NIFA National Needs Fellowship (Grant Nr. 2020-08181).

Graduate Student/BMS

41. Organotypic lung co-culture paradigm to increase T-cell populations ex vivo

Alexis Ehrlich and Stuart Tobet

Precision cut lung slices (PCLS) bridge a gap between in vivo and in vitro studies by maintaining anatomical organization with structural integrity and intercellular signaling pathways. In the lungs, immune responses are carried out by a network of T- and B- cells, the latter of which are resident. However, there is a limited resident T-cell population in PCLS. Addressing this, we set out to increase pulmonary T-cell populations ex vivo. We hypothesized that thymus and bone marrow-derived T-cells would work synergistically to populate the lung in co-culture experiments. A murine organotypic lung co-culture model was developed and characterized for T-cell recruitment over 3 days ex vivo using adult neurobasal media. Lung slices were cultured independently, with bone marrow, thymus, or both. Immune colonization was assessed using immunohistochemistry for CD3+ T-cells and ACK2+ cells. Cells were counted in alveolar and airway spaces after 3 days of culture. Co-culture with bone marrow did not increase CD3+ immunoreactive T-cells while thymus co-culture increased CD3+ T-cells by 76% in the alveolar space and by 39% in the airway, relative to lung alone. When lung slices were cultured with bone marrow plus thymus, CD3+ T-cells increased by 206% in the alveolar space and by 251% in the airway, relative to the lung alone. These results suggest that the increased T-cell population corresponding with thymus and bone marrow co-culture could be a result of cell-cell interaction or the secretion of growth factors. Cell secretions or interactions could stimulate thymic secretion of T-cells or could stimulate T-cell proliferation in the lung, suggesting that co-culture with thymus and bone marrow can elicit a T-cell response ex vivo. Future studies will center around differentiating what drives T-cell population to be increased and what T-cell subsets are being recruited in PCLS co-cultures. Research support provided by the Anschutz Pandemic Preparedness Grant.

Graduate Student/ BMS

42. Testing of Candidate Formulations of CSU's SARS-CoV-2 Vaccine (SolaVAX-CoV-2) for Phase I Human Clinical Trial Use

Arielle Glass, Izabela Ragan, Lindsay Hartson, Nicole Kruh-Garcia, Jordan Flatt, Kaitlyn Szlosek, Darragh Heaslip, Ray Goodrich

The COVID-19 pandemic has intensified the need for rapid development and evaluation of vaccines against both COVID-19 and other emerging pathogens with global impact. Fueled by the urgent need to combat the ever-evolving SARS-CoV-2, novel approaches for vaccine development and immune activation are currently undergoing rigorous scrutiny in clinical settings. We present a novel photochemical method (SolaVAX™) for producing inactivated vaccine candidates and evaluate the immunogenicity of purified formulations in a well-characterized hamster model. Our previous studies in preclinical models examined long-term neutralizing antibody levels post-vaccination, levels of specific antibody subtypes to RBD and spike protein, viral shedding post-challenge, flow cytometric and single-cell sequencing data on cellular fractions, and histopathological evaluation of tissues post-challenge. In this study, we evaluated three different preparations of the new vaccine using different purification methods to identify a candidate that has optimized purity and immunogenicity. We intramuscularly administered two doses for each vaccine formulation to hamsters (N=10/group) and collected blood at 21- and 42-days post-vaccination. Plaque reduction neutralization tests (PRNTs) were performed to assess neutralizing antibody titers, which show neutralizing antibody titers that range from 1:10 to 1:>320 (90% cut off) at 42 post-vaccination. Sample analysis is still ongoing and will be reported upon completion. These preliminary results indicate that the new vaccines are generating strong antibody responses and will be protective against live viral challenge, which will be evaluated in our next study. This data will ultimately guide the manufacturing process for our Phase I clinical trial. The SolaVAX™ photochemical method may hold potential for the rapid preparation of vaccine candidates against neglected and emerging viral pathogens.

Graduate Student/MIP

43. Causes of mortality in sugar gliders (Petaurus breviceps) presented to James L. Voss Veterinary Teaching Hospital, Colorado State University

Lindsey R. Godwin, Sangeeta Rao, Miranda Sadar

Introduction: Sugar gliders (Petaurus breviceps) have grown in popularity as a zoological companion animal species. Thus, this species may be presented to veterinarians for a variety of medical conditions. Little is known about this species, especially the causes of their mortality in captivity. The objective of this study was to describe the causes of mortality of sugar gliders presented to a veterinary teaching hospital in Colorado, United States. Materials and Methods: A medical record database was searched from April 7, 2000 through January 24, 2023. Records from 68 sugar gliders were evaluated. Inclusion criteria were sugar gliders that had presented to the veterinary teaching hospital that had a complete necropsy performed, including histopathology. Results: Data were included from 68 sugar gliders, with 24 males, 13 females, and 31 unknown sexes. Ages at postmortem ranged from 2.85 months to 13.25 years old. The most common causes of mortality included nephritis (13/68, 19%), enteritis and/or colitis (11/68, 16%), and hepatitis (10/68, 15%). Lymphoplasmacytic changes were found in 44/68 individuals. Other causes included respiratory and neurological disease. Discussion: These results showed that stress-induced anorexia combined with systemic inflammation and infection was the leading cause of death. This may be related to suboptimal husbandry and diet in captivity. Future work should focus on increasing data through incorporating multiple institutions across the country. Conclusions: Establishing the leading causes of mortality aids in expanding the knowledge of sugar gliders as pets and may lead to prevention and treatment options that increase their survivability in captivity.

DVM Student/ BMS

44. Extended ICU stays, small-gauge catheters, and multiple placements increase risk of peripheral IV catheter complications in cats hospitalized in the critical care unit

Kyle L Granger Jr, Kristin M. Zersen, Liz-Valérie Guieu

Objective: To identify risk factors associated with peripheral intravenous catheter (PIVC) complications in cats hospitalized in the critical care unit (CCU). Animals: 120 cats admitted to the CCU between October 2022 and September 2023. Methods: This prospective, observational clinical trial was performed at a single veterinary teaching hospital. Cats hospitalized in the CCU for at least 24 hours were evaluated for enrollment. PIVCs were placed following a standardized protocol and monitored for complications. PIVC complications were classified as: extravasation, phlebitis, dislodgement, occlusion, line breakage, or patient removal. Results: Median PIVC dwell time was 42.25 hours (range, 24.25–164.25 hours). Overall PIVC complication rate was 18.3% (21/110), with extravasation (7/120; 5.8%), and dislodgement (7/120; 5.8%) being the most frequently recorded complications. Univariate analysis identified cumulative catheters placed during hospitalization (P = <0.0001), length of hospitalization (LOH) (P = 0.02), and PIVC gauge (P = 0.023) as statistically significant risk factors for PIVC complications. Multivariable analysis identified increasing LOH (OR: 1.45, 95% CI: 1.015, 2.071; P = 0.0412) and having a smaller gauge PIVC (OR: 0.175, 95% CI: 0.046, 0.667; P = 0.0107) were associated with an increased odds of PIVC complication. Clinical Relevance: Increasing LOH, smaller gauge PIVCs, and cumulative PIVC placements during hospitalization as risk factors for PIVC complications in cats hospitalized in the CCU. The persistence of statistical significance of LOH and smaller gauge PIVCs in the multivariable analysis emphasizes reinforces the notion that these variables are not merely correlated but may have a causal relationship with the occurrence of PIVC complications. Cumulatively, our findings accentuate the significance of considering not only specific risk factors but also their interplay in guiding clinical decisions and healthcare practices to optimize patient care and minimize PIVC-related complications. Research support provided by the Jorgensen Fund.

Resident/CS

45. Characterization of exosome labeling techniques and implications for downstream analysis

Laurel A. Haines, Alex A. Baeckler, Sophi J. Schofield, Eric P. Palmer, Aaron D. Offerman, Daniel P. Regan

Extracellular vesicles and their intercellular signaling are a rapidly growing area of study across a range of scientific disciplines, including cancer biology. Among researchers, there remains considerable heterogeneity in the methods used for vesicle isolation, characterization, and labeling. We aimed to develop reproducible techniques for the isolation of a specific subset of nano-sized extracellular vesicles known as exosomes. Moreover, we investigated methods for fluorescent labeling of exosomes for subsequent use in cell-based and in vivo studies. Optimization of exosome handling techniques is critical to the accurate evaluation of the biological impact of these nanoparticles. We isolated exosomes from osteosarcoma bone tumor cell lines using ultrafiltration followed by size exclusion chromatography and evaluated concentration and morphology via NanoSight particle analysis and transmission electron microscopy. These techniques reproducibly generated exosome isolates of high purity as assessed by expression of exosome surface markers. We then used spectral flow cytometry, intravital imaging, and confocal microscopy to investigate exosome labeling methods using the membrane intercalating dye PKH26. Interestingly, we identified specific experimental techniques that can lead to erroneous results during exosome labeling. Specifically, we found that the buffer conditions used to isolate and label exosomes can trigger the formation of dye aggregates that are nearly indistinguishable from exosomes. Through optimizing buffer conditions, we minimized the formation of dye aggregates while maintaining efficient exosome labeling. We also demonstrated that improper labeling techniques can have significant implications for cell-based assays and in vivo imaging, potentially leading to false positive results. The use of precise exosome isolation and labeling techniques is essential for accurate downstream evaluation of their role in tumor biology as well as in other physiologic and pathologic states. Research Support provided by the Morris Animal Foundation, the Boettcher Foundation Webb Waring Biomedical Research Award, National Institutes of Health RO3OD028265, and National Institutes of Health Medical Scientists Training Program T32GM136628.

DVM/PhD Student/ MIP

46. A descriptive study of head and neck tumors in young dogs

Regina Hayburn, Gabrielle Fontes, Laura Selmic, Emma Warry

Background: Oral tumors in young dogs have been poorly described in veterinary literature, with primarily case reports describing head and neck tumors in dogs under 4 years of age. These tumors are believed to have a locally aggressive biological behavior and are thought to be frequently metastatic at the time of diagnosis. Our primary objective was to describe demographics of the affected population, histotypes of malignant tumors diagnosed, treatments utilized, and subsequent outcomes. Methods: Client-owned dogs with a cytologic or histologic diagnosis of a mass in the head or neck were identified by an electronic medical record search from the Colorado State University Veterinary Teaching Hospital, Texas A&M University Veterinary Teaching Hospital, and The Ohio State University Veterinary Teaching Hospital between September 2006 and September 2022. Inclusion criteria consisted of the presence of a mass in the head and neck in dogs under the age of 4 years, with a diagnosis via cytology or histopathology. Cases were excluded if no attempt at diagnosis was performed or if the patient was over four years of age at the time of diagnosis. Results: A total of 31 dogs fulfilled the inclusion criteria. The median age of patients was 655 days. The majority of the tumors were sarcomas (n=13), followed by SCC (n=6), melanoma (n=3), MCT (n=3), and 1 of each poorly differentiated neoplasm, inflammatory disease but suspect neoplasia based on imaging, MLO, fibroma suspect FSA, acanthomatous ameloblastoma, and LSA. The most common location was on the maxilla (n=16). Ulceration was reported in 12/31 (38.7%) histopathology reports. Clinical signs consisting of oral discharge, pain, or difficulty eating were identified in 29/31 (93.5%) cases. Conclusion: Oral sarcomas were found to be the predominant oral tumor of young dogs. The majority of young dogs presenting with these tumors exhibited clinical signs impacting patient quality of life.

Resident/CS

47. Omega-3 fatty acid docosahexaenoic acid inhibits *Staphylococcus aureus* growth mediated by FadA β-oxidation

Julia Hilliard, Casey Gries

Prosthetic joint infection (PJI) represents a key health concern with ~2% of all joint replacements resulting in infection. PJI patients have a 10-year mortality rate that is 16% higher than joint replacement patients without subsequent infection. *Staphylococcus aureus* is a leading causative agent in PJIs with incidence on the rise, including cases of methicillin-resistant *S. aureus* (MRSA) infections. *S. aureus* PJIs often result in biofilm formation; a bacterial growth state highly tolerant to antibiotic treatments. As such, improved treatment options are necessary for successful amelioration of *S. aureus* PJI. Recently, emerging evidence have indicated potential antimicrobial and antibiofilm effects of the omega-3 fatty acid docosahexaenoic acid (DHA) against *S. aureus*. DHA is acquired through diet and is essential for brain development and function. It is also a precursor to pro-resolving lipid mediators that promote inflammation resolution and repair during immune response. However, the mechanisms by which DHA impacts *S. aureus* growth are not understood. Here, we screened a sequence-defined transposon mutant library of 1,920 MRSA strains for the ability to grow in 250 μM DHA. Mutants which grew above a set threshold in the presence of DHA were confirmed and selected for further study. A mutation in fadA, which is part of the fadXDEBA locus in *S. aureus* and responsible for the β-oxidation of fatty acids, was identified. The ability of the fadA mutant to proliferate in the presence of inhibitory DHA concentrations suggests a mechanism wherein DHA disrupts fatty acid metabolism via altering the production of acetyl-CoA, a product of FadA activity. This disturbance of normal stoichiometric acetyl-CoA concentrations may lead to activation of a σB stress response resulting in loss of *S. aureus* viability. Proper function of the β-oxidation pathway is important for *S. aureus* growth and demonstrates a possible target for DHA's antimicrobial activity.

Graduate Student/ MIP

48. Bluetongue virus surveillance across domestic ruminants in Northern Colorado in 2022

Samantha Hilty, Mollie Burton, Christie Mayo

Bluetongue virus (BTV) poses a significant threat to both wild and domestic ruminants worldwide. Transmitted by the *Culicoides* biting midge, outbreaks of BTV in domestic ruminant populations can cause substantial agricultural and economic losses on a global scale, with estimated annual impacts reaching up to 3 billion US dollars. Surveillance of bluetongue virus is essential for monitoring the spread of bluetongue disease and implementing management strategies to help mitigate its impact. This study aims to assess the seroprevalence of bluetongue virus in domestic ruminants in Northern Colorado. Serum samples were collected monthly from May 2022 to December 2022 from domestic sheep, dairy cattle, and beef cattle representing 8 sites and 169 unique animals. The serum samples were screened for the presence of bluetongue virus-specific antibodies using competitive enzyme-linked immunosorbent assay (cELISA). Overall seroprevalence in June was estimated to be 54.6%, with the final seroprevalence in December estimated to be 56.3%. The estimated BTV seroprevalence for the duration of the collection period was 56.9% for sheep sites, 82% for beef sites, 38.5% for dairy sites, and 69.1% for all sites combined. These results underscore the ongoing presence of bluetongue virus in Northern Colorado and the importance of supporting surveillance efforts to inform targeted control strategies to effectively manage bluetongue in Northern Colorado. Research support was provided by United States Department of Agriculture Fellowship, USDA-NIFA AFRI grant number 2019-67015-28982 as part of the joint USDA-NSF-NIH-BBSRC-BSF Ecology and Evolution of Infectious Diseases program. Student support was provided by USDA-NIFA-ADHR-COLV-2023.

DVM Student/MIP

49. Jamaican fruit bats and sarbecovirus susceptibility

Natasha Hodges, Tony Schountz

SARS-CoV-2, the etiological agent of the COVID-19 pandemic, is among many SARS-related coronaviruses in the subgenus *Sarbecovirus*, genus *Betacoronavirus*. SARS-CoV-like variants related to SARS-CoV-2 have been found in found in *Rhinolophus spp*. bats, including RmYN02 (93.3%), RmYN06 (94.4%), RaTG13 (96.1%), and BANAL-52 (96.8%) (percent identity at whole-genome level). BANAL-52 and BANAL-236 are viruses of interest because of the high similarity at the whole genome level and because BANAL-52 shares 16/17 residues that interact with human ACE2 (hACE2) and for BANAL-236, 13/17 residues. Both viruses have the ability to bind to hACE2. For these reasons, sarbecoviruses originating in bats are hypothesized to be SARS-CoV-2 progenitor viruses and may still pose future risk. Understanding the diversity of bat coronaviruses is crucial to predicting zoonotic potential. Current data show that Jamaican fruit bats (*Artibeus jamaicensis*) are poorly susceptible to SARS-CoV-2 (WA-1 isolate), but that expression of hACE2 in their lungs lead to infection and adaptive immune response. Burke *et al.* show inoculated Jamaican fruit bats with a replication-defective adenovirus encoding human ACE2 and express hACE2 for up to 21 days. First, we will determine if Jamaican fruit bats are susceptible to BANAL-CoV's (BANAL-52/BANAL-236). Secondly, we will determine susceptibility, infection dynamics, and pathology of *A. jamaicensis* bats transduced with hACE2. Like the aforementioned study, we suspect to see poor susceptibility with inoculation alone, however, we anticipate that we will see susceptibility and pathology with the transduction of hACE2. This data will help establish whether BANAL-CoV's cause infection of Jamaican fruit bats and thus show the ability for these bats as a model animal for future therapeutic studies. Student support provided by IMSD T32 Training Grant T32GM144856.

Graduate Student/ MIP

50. Efficacy of a combination of selamectin + sarolaner placed on cats for the prevention of transmission of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* from infected Ixodes scapularis

Rae Isdale, Kaci Shaw, Jennifer Hawley, Michael Lappin

Borrelia burgdorferi (Bb) and *Anaplasma phagocytophilum* (Ap) are vectored by *Ixodes scapularis* and are associated with fever and other signs in some cats. The combination of selamectin + sarolaner (Revolution Plus; Zoetis) applied topically significantly reduces *I. scapularis* on cats by 12 hours with 100% efficacy by 24 hours. In previous studies, it has been shown that a commercially available kit (SNAP 4DxPlus; IDEXX) for detection of antibodies against Bb and Ap in dog sera can be used with feline sera. The objective of this study was to determine if RP protects cats against transmission of Bb and Ab from wild caught *I. scapularis* by killing the ticks before disease transmission can occur. A total of 20 mixed sex, young adult cats were shown to be negative for Bb and Ap antibodies before being randomized into two groups of 10 cats treated with RP or placebo on Day 0. Wild caught adult *I. scapularis* (Rhode Island) infected with both Bb and Ap were used to infest the individually caged cats by dropping 25 male and 25 females onto the dorsal head and thorax while sedated for Elizabethan collar placement on Day 30. Tick counts and removal of ticks and collars were completed on Day 35. The cats were housed so that other cats could not be touched, and protective equipment was changed between cats to avoid transfer of RP to placebo cats. Blood samples for serology were collected after infestation and by Day 104, antibodies against Ap (4 cats) or Bb and Ap (5 cats) were detected in control cat sera. In contrast, none of the RS treated cats developed Bb antibodies and Ap antibodies were only detected in 1 cat. Results suggest that risk of infection by both agents can be reduced RP. Research supported by Zoetis Animal Helath and the Center for Companion Animal Studies.

DVM Student/CS

51. Effects of an innate immune stimulant on dairy calf respiratory health, Salmonella shedding, and cytokine gene expression

Grace M. Jakes, Lance D. Sommer, Jenna N. Gotte, Tanya J. Applegate, Steven Dow, Sarah Raabis

Bovine respiratory disease (BRD) is a leading cause of morbidity and mortality in preweaned dairy calves. BRD has also been linked to an increased risk of Salmonella spp. shedding. While antimicrobials are commonly administered, alternative treatment strategies are needed to reduce antimicrobial use. Innate immune stimulation using toll-like receptor (TLR) agonists has emerged as a strategy to reduce BRD incidence, but the prophylactic effects of this treatment have not been evaluated in pre-weaned dairy calves. The objectives of this study were to evaluate the effects of an innate immune stimulant on dairy calf respiratory health, cytokine gene expression and *Salmonella spp*. shedding. 50 pre-weaned Holstein calves were assigned to one of two intranasal dosing groups administered at 1, 2, and 3 weeks of age. Control: 2 mL diluent (n=25); LTC: 0.1 mL liposome TLR complex (LTC) in 1.9 mL diluent (n=25). Clinical and ultrasound scores were collected twice per week using the University of Wisconsin-Madison calf health scoring system up to 10 weeks of age. Deep nasopharyngeal swabs (DNPS) were collected on each calf pretreatment, and at 2, 3, 4, and 8 weeks of age. Additionally, fecal cultures were performed pre-treatment and at 2, 4 and 8 weeks of age. Cytokine gene expression from DNPS was evaluated using RT-qPCR for the bovine cytokines INF-γ and MCP-1. There were no differences in the odds of developing clinical or subclinical respiratory disease between treatment and control groups. Incidence of subclinical respiratory disease was higher than clinical disease presentation, but both rates were relatively low (20% and 32% respectively) in this population. Salmonella shedding was not linked to respiratory disease or to LTC treatment. These results suggest that innate immune stimulation may not improve respiratory disease risk in healthy pre-weaned calves, or that calves may have been overstimulated by the LTC treatment. Research support was provided by a CVMBS CRC award. Student support provided by National Institutes of Health Medical Scientists Training Program T32GM136628.

DVM/PhD Student/ CS

52. Deciphering the interplay of IL-22 and IL-22BP in placental development: implications for maternal-fetal immunomodulation

Kaylee Jones, Tara M. Nordgren

The growth and survival of the fetus is reliant on the placenta for providing oxygen and nutrients during pregnancy. The delicate balance between immune tolerance and pathogen defense can challenge placental integrity and fetal survival. Interleukin-22 (IL-22) has both pro-inflammatory and pro-resolution functionalities for homeostatic control. Regulation of IL-22 occurs through binding and sequestration via the inhibitory IL-22 binding protein (IL-22BP). As such, IL-22 and IL-22BP have risen to become a subject of interest in maternal-fetal immunomodulation during varied gestational stages. This study aims to understand the localization and compartmentalization patterns of IL-22 and IL-22BP across gestation. We hypothesize that IL-22BP levels are increased during fetal development, which will serve to inhibit maternal IL-22 immune system responses to the placenta and fetus and ensuring a successful pregnancy. Via immunohistochemistry (IHC) of postnatal human placental tissues, we aim to correlate IL-22 and IL-22BP's spatiotemporal nature with clinical gestational features. These outcomes will allow for an increased understanding into the pivotal roles of IL-22 and IL-22BP and their control in fetal and maternal health outcomes. This work was supported by the NIH/NHLBI via R01HL185926 to Tara M. Nordgren.

Graduate Student/ERHS

53. Characterizing the nicotine metabolite ratio and its association with sociodemographic and smoking characteristics in HIV-infected smokers in south africa

Chukwudi Keke, Zane Wilson, Limakatso Lebina, Katlego Motlhaoleng, David Abrams, Ebrahim Variava, Nikhil Gupte, Raymond Niaura, Neil Martinson, Jonathan E Golub, Jessica L Elf

The nicotine metabolite ratio (NMR) has demonstrated potential in selecting treatment choices to improve smoking cessation but has not been evaluated among smokers in the African region. We conducted a cross-sectional analysis of baseline data from a large randomized, controlled trial for smoking cessation among people with HIV (PWH) in South Africa. Urine samples were analyzed for the NMR and evaluated as a binary variable using a cutoff value of the fourth quartile to determine the fastest metabolizers. The median NMR was 0.31 (IQR: 0.31, 0.32; range: 0.29, 0.57); the cut-point for fast metabolizers was ≥0.3174 ng/mL. A high NMR was not associated with the number of cigarettes per day (OR = 1.10, 95% CI: 0.71, 1.70, p = 0.66) but was associated with 40% lower odds of a quit attempt in the past year (OR = 0.69; 95% CI: 0.44, 1.07, p = 0.09) and alcohol use (OR = 0.59, 95% CI: 0.32, 1.06, p = 0.07). No association was seen with marijuana or HIV clinical characteristics. As we found only minimal variability in the NMR and minimal associations with the intensity of smoking, NMR may be of limited clinical value in this population, although it may inform which individuals are less likely to make a quit attempt. This research was funded by the National Institute on Drug Abuse of the National Institutes of Health, grant number R01DA030276 and the College Research Council of the College of Veterinary Medicine and Biomedical Sciences at Colorado State University.

Graduate Student/ ERHS

54. A Habitat Suitability Analysis for Three Culicoides Species Implicated in Bluetongue Virus Transmission in the Southeastern United States

Peter J Kessinger, Angela James, Kelly A. Patyk, Stacey L. Vigil, Mark G. Ruder, Sheryl Magzamen

Culicoides biting midges adversely impact animal health through transmission of multiple orbiviruses such as bluetongue virus (BTV). This study used trapping data collected in the Southeastern United States for three Culicoides midge species that are potentially competent BTV vectors: *C. insignis*, *C. stellifer*, and *C. venustus*. Midge presence datasets were combined with meteorological data, soil data, and normalized difference vegetation index to characterize midge populations, and describe habitat suitability for each species. Logistic regression and machine learning models were used to generate individual species distribution models (SDM). Results for each SDM method were combined in an ensemble model to create a distribution model for each midge species. Based on overlay analyses of livestock populations and midge suitable habitat, there is extensive overlap of cattle and goat populations in Florida and *C. insignis*' suitable habitat. *Culicoides stellifer* suitable habitat intersects with cattle and goat populations in various counties in Alabama, Arkansas, the Carolinas, Florida, Georgia, Louisiana, and Tennessee; and *C. venustus'* suitable habitat intersects with cattle and goat populations in the same states as *C. stellifer* except for Florida. Orbivirus and midge surveillance are critical to continue in the Southeastern United States with all three midge species' habitats intersecting with livestock populations. Research supported by USDA Cooperative Agreement AP21VSSP0000C073.

Graduate Student/ERHS

55. Axonal Transport of Alpha-Synuclein Prions

Chase R. Khedmatgozar, Amanda L. Woerman

Synucleinopathies, which include Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy, are caused by the protein alpha-synuclein misfolding into distinct conformations, or strains. Each α-synuclein strain propagates from cell-to-cell using the prion mechanism of disease, which enables progressive spread throughout the central nervous system. For this to occur, α-synuclein prions must be taken up by a neuron, where replication occurs before newly synthesized prion is released to infect the next neuron in a circuit. While much is known about both the uptake and release mechanisms involved in spread, it remains unclear how α-synuclein prions are transported within the neuron. Moreover, given that each strain differs in its biophysical properties, including aggregate size, it is possible that strain-specific differences in the rate of transport contribute to the unique disease pathogenesis associated with each synucleinopathy. To investigate this mechanism, I am developing a mouse primary neuron model using P0 transgenic mice expressing human α-synuclein with the A53T mutation. To date, isolated cortical neurons have been infected with recombinant α-synuclein fibrils over a time course of 21 d. Using an AlexaFluor dye to tag each α-synuclein prion strain will enable unbiased quantification of axonal transport. By understanding how α-synuclein transport occurs, I expect to identify possible therapeutic targets that prevent the spread of disease in synucleinopathy patients. The Woerman Lab is supported by the Rainwater Charitable Foundation, Colorado State University, and NIH grants NS121294, NS121294-S1, and NS127002.

Graduate Student/ MIP

56. Co-activation of selective nicotinic acetylcholine receptors improves hippocampal brain rhythms and memory in the mouse of Alzheimer's disease

Rahmi Lee, Seonil Kim

Different subtypes of GABAergic inhibitory interneurons produce hippocampal oscillations where reduced activity in these interneurons is linked to lower oscillatory activity and memory loss in AD. In the early stages of AD, beta-amyloid peptide (Aβ) is linked to decreased hippocampal oscillations due to decreased GABAergic inhibition, resulting in cognitive impairment, the mechanism however is unknown. A prominent AD pathology in the human brain is the loss of cholinergic neurons and nicotinic acetylcholine receptor (nAChR) expression. Our findings show that co-activating the subtypes α7- and α4β2-nAChRs in vivo improves memory in an AD mouse model. We hypothesize that Aβ reduces hippocampal GABAergic activity by selectively inhibiting α7- and α4β2-nAChRs, resulting in hippocampal oscillation disruption and memory loss in AD, and that selective co-activation reverses the Aβ-induced pathological effects. The AD mouse model, 5XFAD transgenic mice, with wild type (WT) littermates treated intraperitoneally with α7- and α4β2-nAChR agonists 1 μM PNU-282987 and 2 μM RJR-2403 Oxalate respectively at concentrations of 5mg/ml for 7 days. We compare both mice models with same volume of 0.9% saline as control. We performed stereotaxic surgery to insert electrodes into CA1 of hippocampus to measure local field potential of theta and gamma oscillations. At control conditions we found that 5XFAD and WT mice had similar power spectrum density (PSD). After consolidation there was definite decrease in PSD for 5XFAD mice. Then we found that with co-stimulation of nAChRs we can rescue the PSD. We also performed fear conditioning to see if memory consolidation increases with dual injection of the agonists. We observed that with control conditions 5XFAD has clear deficit in contextual memory which is then successfully rescued by co-activation. Research support provided by NIH, The Boettcher foundation, and The BrightFocus Foundation.

Graduate Student/BMS

57. Inhibition of two inflammatory pathways protects from pathological changes due to neurotoxin, MPTP, induced Parkinson's disease

Avery Lessard, Sydney Risen, Yoselyn Gonzalez, Prashant Nagpal, Mark Zabel, and Julie Moreno

Parkinson's disease (PD) is a neurodegenerative disease that primarily effects the substantia nigra pars compacta brain region and the neurotransmitter dopamine. Inflammation and the accumulation of the misfolded protein alpha-synuclein are known to occur as the disease progresses. To study the neuroinflammation detected in PD, we utilized the commonly used neurotoxicant experimental MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model to test if inhibition of two common inflammatory signaling events would prevent disease progression. Using this model, we exposed MPTP mice to Nanoligomers™ both interperitoneally and intranasally twice a week to inhibit both NF-kB and the inflammasome; NLRP3. Following four weeks of treatment, we assessed through immunohistochemistry to determine whether Nanoligomers™ modulated the MPTP induced glial inflammation by counting numbers of microglia and astrocytes. The results indicate that there is significant reduction in the number of microglia and astrocytes that were induced by MPTP treatment, indicating that these two inflammatory pathways reduce MPTP induced neuroinflammation. We then identified that MPTP-induced neuronal loss in the SNpC was also inhibited by treatment with Nanoligomers™. Throughout our analysis internasal delivery of the Nanoligomers™ appears to be more effective than the intraperitoneal delivery. In conclusion, the inhibition of specific inflammatory markers through drug treatment appears to be successful as preserving neuronal cells and decreasing inflammation. The results indicate that the inhibition of NF-kB and the inflammasome complex, NLRP3, is neuroprotective in a neurotoxin mouse model of PD. Student supported by the NIH PREP award number R25GM148297.

PostBacc/ ERHS

58. Characterizing tuberculosis immunopathogenesis in guinea pigs: validation of antibodies for cytokine profiling

Alyssa Longworth, Lea Maristela, Tori Mitcham, Forrest Ackhart, Faye Lanni, Brendan Podell

The guinea pig model of *Mycobacterium tuberculosis* (*Mtb*) offers distinct advantages compared to conventional mouse models. Namely, the guinea pig model develops circumcised granulomas with central necrosis which most closely resembles that of human granulomas. However, a limitation of this model is the availability of reagents to characterize the *Mtb* immune response. To better leverage this representative model, we evaluated a set of rabbit monoclonal antibodies produced through molecular cloning of the B cell receptor from antigen-specific B cells in the peripheral blood of rabbits immunized with full length recombinant guinea pig cytokines generated in HEK293 cells. Antibodies were produced through molecular constructs transfected in HEK293 cells. We have established stimulation conditions for primary guinea pig cells, including PBMCs, splenocytes and bone marrow-derived macrophages, capable of producing specific cytokine profiles. Antibody specificity for the detection of native protein was determined by immunoprecipitation followed by MS-MS sequence confirmation, prior to the validation of functional antibody pairs to be used in capture ELISA and ELISpot techniques or evaluated individually as reagents for flow cytometry. Utilized in a low-dose aerosol model of TB in the guinea pig, these reagents will provide a more comprehensive understanding of immune kinetics, vaccine response, and comparative immunology across TB model species. Research support provided by NIH R01AI162746.

DVM Student/MIP

59. Using real-time cognitive load measurement to assess instructional design effectiveness

Brandon L. Lowry, Chad Eitel, Samantha McGrath, Becky Wiltgen, Kenneth Ivie, Jr., Carolyn Meyer, Brian Kelly, Heather Hall, Tod R. Clapp

Integrating virtual reality (VR) into education can transform teaching and learning allowing users to interact with three-dimensional data similar to how it exists in nature. Instructors across disciplines use VR to innovate and overcome traditional education barriers. However, there is a significant need for evidence-based resources for VR-based instructional design to ensure effective implementation. This project seeks to establish instructional design practices for VR that optimize cognitive load, enhance learning, and improve the learner's experience. A mixed-factorial research design was used to assess differences and associations among undergraduate students between (n = 91) and within (n = 43) groups. Our objectives include evaluating cognitive load differences based on data type, assessing the impact of content sequencing on cognitive load, and exploring the interaction between student attitude/behavior and cognitive load. Combining pupillometry, eye gaze, and heart rate biometric data, we utilized biometrics to define these real-time measurements as an indicator of cognitive load. Analyses included independent and paired t-tests, single and multi-factor ANOVA, and linear regression. Results indicate statistically significantly lower mean cognitive load for students viewing 3D content compared to 2D content in between-group, $t(41) = 4.9$, $p < 0.001$, and within-group comparisons, $t(90) = 10.83$, $p < 0.001$. Attitude toward virtual reality did not statistically significantly impact mean cognitive load ($F(2, 87) = 0.521$, $p = 0.596$). Conversely, content sequencing significantly influenced cognitive load ($F(1, 84) = 4.430$, $p = 0.038$). The data demonstrates that virtual learning experience design and data visualization can manipulate cognitive load, while student attitude has limited impact on instructional design effects. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. 2234690.

Graduate Student/ BMS

60. Infralimbic prefrontal cortical projections to the autonomic brainstem: quantification of inputs to cholinergic and adrenergic/noradrenergic nuclei

Ema Lukinic, Tyler Wallace, Brent Myers

The ventromedial prefrontal cortex regulates both emotional and physiological processes. In particular, the infralimbic cortex (IL) integrates behavioral, neuroendocrine, and autonomic responses to stress. However, the organization of cortical inputs to brainstem nuclei that regulate homeostatic responses are not well defined. Therefore, we hypothesized that IL projections differentially target pre-ganglionic parasympathetic neurons and adrenergic/noradrenergic nuclei. To quantify IL projections to autonomic brainstem nuclei in male rats we utilized viral-mediated gene transfer to express yellow fluorescent protein (YFP) in IL glutamatergic neurons. YFP-positive projections to cholinergic and adrenergic/noradrenergic nuclei were then imaged and quantified. Cholinergic neurons were visualized by immunohistochemistry for choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine. Adrenergic/noradrenergic neurons were visualized with immunohistochemistry for dopamine beta hydroxylase (DBH). DBH converts dopamine to norepinephrine, which also serves as a precursor for epinephrine. Our results indicate that IL glutamate neurons innervate the cholinergic dorsal motor nucleus of the vagus with greater density than the nucleus ambiguus. Furthermore, numerous DBH-positive cell groups receive IL inputs. The greatest density was to the C2 and A2 regions of the nucleus of the solitary tract with intermediate levels of input to A6 locus coeruleus and throughout the C1 and A1 regions of the ventrolateral medulla. Minimal input was present in the pontine A5. Additionally, we found that these cortical synapses also target local GABA neurons that regulate the identified excitatory neurons, suggesting potential bidirectional control. Collectively, our results indicate that IL projection neurons target vagal preganglionic parasympathetic neurons, presympathetic neurons of the ventrolateral medulla, as well as diffuse modulators of homeostatic function that arise from the nucleus of the solitary tract and locus coeruleus. Ultimately, these findings provide a roadmap for determining circuit-level mechanisms for neural control of homeostasis and autonomic balance. Research support provided by NIH R01HL150599.

Research Associate/BMS

61. Inactivated Mycobacterium tuberculosis as a vaccine strategy for Tuberculosis disease

Pablo Maldonado Jr., Taru S. Dutt, Lindsay Hartson, Raymond Goodrich, Izabela Ragan, Olivia Asfaha, Michael Artinger, Andres Obregon Henao, Marcela Henao Tamayo

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains a significant global health concern with 1.4 million reported deaths in 2021. The Bacillus Calmette Guérin (BCG) vaccine is the only licensed vaccine. However, BCG's protection is not long lived, and variable efficacy may be due to factors such as strain variability and the absence of Mtb-specific antigens. Various vaccine approaches, including subunit, live attenuated, and heat-inactivated vaccines, have been explored by researchers, yet challenges persist in inducing Mtb specific immunity. In response to these challenges, the SolaVAX-Mtb vaccine booster has been developed utilizing the SolaVAX technology at Colorado State University. This technology employs riboflavin and UV light to inactivate Mtb, disrupting its genetic material while maintaining antigen integrity. SolaVAX offers advantages such as whole-pathogen antigen presentation, rapid manufacturing, cost-effectiveness, and versatility across pathogens. This study seeks to validate SolaVAX-Mtb by assessing its inactivation, safety, immunogenicity, and efficacy when administered as a booster to BCG. Results indicate that SolaVAX-Mtb remains immunogenic post-genetic disruption and is safe in immunocompromised mice. Moreover, when administered as a BCG booster, SolaVAX-Mtb demonstrates a reduction in bacterial burden compared to BCG alone. The study also identifies potential correlates of protection, with SolaVAX-Mtb activating significant CD44+ dendritic cells in the bronchoalveolar lavage fluid of Mtb-infected mice as well as elevated levels of B-cells in the lungs. This research contributes to the development of a reliable, safe, and effective inactivated whole-cell Mtb vaccine and addresses the pressing need for enhanced TB prevention strategies on a global scale.

Graduate Student/ MIP

62. "The most important thing is to know what to wear when working in the sun": Crop workers perspectives on workwear.

Yessica P. Martinez, Whitney Pennington, Kayna Murphy-Hobbs, Morgan Valley, John Rosecrance

Crop workers face increasingly hot working conditions. Wearing light-colored, single-layer breathable clothing and wide-brimmed hats, on top of having water, rest, and shade, can reduce the risk of heat illness among crop workers. Clothing preferences and limited access may prevent crop workers from wearing optimal work apparel. However, little is known about the crop workers' workplace clothing preferences or their experiences acquiring work clothing. Objective: This qualitative study aimed to learn Spanish-speaking crop workers' ideal work clothes and understand the primary barriers to wearing clothes that reduce heat-related illness risks. Methods: Four focus groups were conducted in Spanish on vegetable farms in Colorado during August and September 2023. Two researchers thematically analyzed detailed focus group notes. Results: Fifty-four Spanish-speaking crop workers participated in focus groups during August and September 2023. Findings show that participants know which work clothing options protect from heat-related illnesses. Participants described purchasing their work clothes at second-hand and discount stores. They preferred clothes made of light but durable materials and complete pieces that cover the head, neck and trunk with long sleeves and pants that facilitate movement. Barriers to wearing ideal clothing described include costs and the difficulty in finding sizes adapted to Hispanic anthropometry among the Anglo-Saxon market. Participants described an interest in partnering with employers to purchase ideal clothing to prevent heat-related illness. Conclusions: Spanish-speaking crop workers have a clear understanding of ideal work clothing and the link between clothing and heat illnesses prevention. However, cost and access to ideal clothing in appropriate sizes are the biggest barriers. In the future, it would be ideal to find strategies that can facilitate access to appropriate clothing to prevent heat illnesses and possible involving of employers seen as a potential access bridge, promoting worker safety as a pillar of agricultural companies. Research support provided by CDC/NIOSH Grant No. U54OH008085.

Graduate Student/ERHS

63. Building a one health differential diagnosis: a comprehensive framework for healthcare providers

Megan Mazzotta, KC Hummer, Anuja Riles, Marta Rowh

The One Health approach acknowledges that human, animal, and environmental health are inextricably linked. Currently, One Health is a neglected topic within human clinical practice and medical education. Maintaining individual and global health has become increasingly difficult as societal and environmental challenges such as climate change, the spread of zoonotic disease, loss of biodiversity, pollution, natural disasters, war, and nutrition insecurity continue to threaten well-being. Thus, it is imperative that healthcare professionals are trained to both evaluate and respond to patient presentations through the lens of One Health. A review of literature was performed analyzing the current methods proposed to build a One Health differential diagnosis. We created two human health cases for medical education representing environmental toxin exposure and zoonotic transmission. Through these cases, learners evaluate conditions from a One Health differential. It was determined that when developing a One Health differential, one must consider the impacts of a patient's natural environment (water, air, weather, climate), built environment (housing, transportation, infrastructure), work environment, zoonotic exposures, and companion animals. This framework can guide history taking to form a comprehensive understanding of the patient's condition. Healthcare professionals must be prepared to incorporate the One Health approach in the work-up of disease with animal and/or environmental exposures. We created an infographic and framework to guide history-taking and differential diagnosis generation. Future investigation should be done to evaluate the barriers to the implementation of the One Health model in clinical settings and medical education.

Medical Student/ CU Anschutz

64. Elucidating the microcircuitry of the periaqueductal gray

Kathryn A McCabe & Christopher E Vaaga

The periaqueductal gray (PAG) is a multi-columnar midbrain region that organizes innate defensive behavior strategies in mice. When presented with an ethologically-relevant stimulus, such as a predator, mice will typically engage in either freezing or flight behavior depending on the proximity and environmental resources (such as a nest) available to them. The anatomically distinct columns of the PAG have traditionally been thought to operate primarily independently of one another; with extrinsic synaptic connections from distinct limbic regions of the brain. Here, we challenge this assumption using ex-vivo slice electrophysiology to show bidirectional glutamatergic and GABAergic synaptic connections between two columns within the PAG: the dorsomedial column of the PAG (dmPAG) and ventrolateral column of the PAG (vlPAG), each of which subserve distinct behavioral responses. In both directions, inhibition was stronger than excitation, suggesting the two columns predominately inhibit one another. Additionally, we show that the central amygdala (CeA), a brain region well known for its role in fear, valence, and emotional state regulation, sends direct synaptic connections onto a subpopulation of glutamatergic, freezing-related cells in the vlPAG. Interestingly, both excitatory and inhibitory events were recorded from the amygdala, which is thought to predominately inhibit glutamatergic neurons in the vlPAG. Elicited postsynaptic potentials were recorded and analyzed using parametric and nonparametric statistical analysis and ANOVA. These findings raise the question of how the PAG is integrating both intrinsic and extrinsic synaptic connections in relation to innate defensive behavior and freezing responses, which may serve to elucidate the underlying circuit mechanisms of ethologically relevant defensive behaviors. Research supported by investigator start-up funds.

Research Associate/BMS

65. Cerebellar Vermis Stimulation in Real-Time and Conditioned Place Preference Contexts

Raven A. McGann, Christopher E. Vaaga

The cerebellum is classically considered a motor structure, however increasing evidence in both humans and animal models suggest additional roles in limbic-related, autonomic, and fear-learning and memory functions. In fact, neuroanatomical tracing studies in humans show connections between the medial cerebellar nucleus (mCbN in rodents, fastigial nucleus in primates) to structures including the hippocampus, periaqueductal grey, and amygdala. Additionally, patients with lesion or hypoplasia of the cerebellar vermis display a constellation of affective and cognitive dysfunctions, which has been termed Cerebellar Cognitive Affective Syndrome (CCAS). CCAS patients suffer from a wide range of symptoms including dysregulation of affect, distractibility, impulsiveness, and/or aggressive behavior, suggesting a cerebellar role in emotional and cognitive regulation. Previous work in the lab suggests that disruption of cerebellar activity in mice, in conjunction with a looming visual stimulus (to elicit innate fear responses), results in decreased fear responses and disrupts the normal habituation pattern seen in wild type mice. These results motivate testing whether disruption of mCbN activity directly results in an aversive behavioral response, independent of an innate fear paradigm. To evaluate this, we implanted an optic cannula at the level of vermal Purkinje cells in L7-ChR2 mice, and optically stimulated this region in both conditioned place preference (CPP) and real-time place preference (RTPP) paradigms. Our results indicate that disruption of cerebellar activity drives near complete aversion, which persists in conditioned recall trials and does not readily reverse. Together, these data indicate that disruptions in cerebellar activity is strongly aversive, and further supports the view that the cerebellum plays important roles in fear and emotional processing. Research supported by NIH R00NS119783.

Graduate Student/ BMS

66. Investigating the non-conducting role of K_V2 at ER-PM junctions, a combined *in vitro* and *in vivo* approach

Arielle Michaelis, Josiah Quinn, Michael Tamkun, Frederic Hoerndli

Voltage-gated potassium channels (KV) are widely expressed in the central and peripheral nervous system, and are essential for signal transduction between neurons. KV2 channel disfunction in humans is associated with neurological disorders like epilepsy, ataxia, and intellectual disability. Historically, KV2 has been studied for its voltage-dependent role, in which the channel opens when a neuron is depolarized, resulting in the efflux of K^+ from the cell and restoration of the resting membrane potential. However, the Tamkun lab identified an additional, non-conducting role of KV2 that may be just as important as the channel's voltage-dependent function. The Tamkun lab found that KV2 channels embedded in the plasma membrane (PM) bind to VAP proteins in the endoplasmic reticulum (ER), forming ER—PM junctions. It is well known that the ER plays a crucial role in protein trafficking and in modulating Ca^{2+} signaling that underlies synaptic plasticity. AMPA receptors, crucial for excitatory signaling in neurons, are one such protein that is trafficked through the ER. AMPARs rapidly diffuse throughout the ER, but become concentrated at dendritic spines due to increased ER complexity. Dynamic ER protrusions into mammalian neuron spine heads have been found to be necessary for long-term potentiation, depression, and spatial learning. Preliminary data by the Tamkun lab and the Hoerndli lab suggest that the KV2-ER/PM junctions may play a crucial role in regulating cytosolic and ER Ca^{2+}, as well as exocytosis of AMPARs. We are pursuing the question of whether or not KV2-ER/PM junctions regulate dendritic AMPAR trafficking and delivery in an evolutionarily conserved manner. To do this, we are using *in vitro* and *in vivo* models in tandem. Combining vertebrate cell culture experiments with studies in live *C. elegans* will allow us to pursue a wide variety of questions, ranging from protein functional homology to learning and memory assays. Research supported by NIH R01NS115947.

Graduate Student/BMS

67. Evaluation of aging and skeletal maturity of New Zealand white rabbit: a retrospective radiographic analysis

Bridget Michalko, Lindsey Burton, Brad Nelson, Jeremiah Easley, Katie Sike

Rabbits are a preferred orthopedic preclinical model due to their size, availability, ease of handling, and shorter growth period. Previous studies have reported that rabbits reach skeletal maturity between 7-11 months of age, however, this wide range is dependent on sex and supplier, and makes it difficult to determine the best age of animals to utilize for studies. The study aim was to analyze pre-operative ventral-dorsal radiographs of rabbits sourced from Western Oregon Rabbit Company to determine the age that maximal, steady-state femoral length is reached representing skeletal maturity. To measure femoral length, ImageJ software was used to measure the distance between three separate sets of landmarks used to define 1) mechanical long axis (MLA), 2) proximal/deep femoral length (DFL), and 3) proximal/cranial femoral length (CFL). Measurements were calibrated by setting a scale in ImageJ using a standard ruler included in each radiograph, and sorted based on animal age (5-15 months). Measurements were compared using a One-Way ANOVA with Tukey's Post-Hoc tests (GraphPad Prism 8.3.0). A p-value of less than 0.05 was set as significant for all comparisons. Overall, there was large variability in femoral length at each of the analyzed age points which highlights animal specific variability common with preclinical models. While the three separate measurements each showed significant differences over time (MLA p=0.0127, DFL p=0.0302, CFL p=0.0204), only the MLA showed post-hoc significance between 7 and 11 months (p=0.0415) and 9 and 11 months (p=0.0493). Given the high variability in measurements, and non-linear changes in measurements over time, this suggests that skeletal maturity is stabilized within 5 to 15 months of age. An ongoing prospective study is aimed at obtaining longitudinal monthly radiographs of male and female NZW rabbits from 2 to 12 months of age to further validate skeletal maturity in NZW rabbits.

DVM Student/ CS

68. Spatiotemporal Distribution of Metals in Fine Particulates across the Denver Metro Area

Anne Mielnik, Grace Kuiper, Sherry WeMott, Sheena Martenies, William Allshouse, Christian L'Orange, Anne Starling, John Adgate, Dana Dabelea, Sheryl Magzamen

Metal elements adsorbed to fine particulate matter (PM2.5) are associated with adverse health effects, even at low concentrations in ambient air, yet are difficult for risk assessment due to varying biological responses to species and uncertainties in exposures from atmospheric transport and other meteorological factors. To identify sources of and characterize PM2.5 in the Denver Metro Area, we evaluated spatiotemporal distributions of 15 metals during the Environmental Influences on Child Health Outcomes (ECHO) prebirth cohort study. We used personal air samplers with gravimetric analysis and x-ray fluorescence to quantify PM2.5 and metals, respectively. Spatial variables from regional models (i.e., regionally relevant sources, distance to major roads, presence of wildfire smoke, etc.) and meteorology were also considered. Results indicate concentrations of 13 metals significantly increase with decreases in barometric pressure and temperature, and are highest during wintertime, indicating impacts of inversion ($p < 0.05$). As, Cr, Fe, Ti & Zn concentrations significantly decrease with increasing wind speed ($p < 0.05$), whereas Cu, Ga & Se decrease, but not significantly. Other species are not affected. Cr, Cu, Fe, K, Ti & Zn significantly increase nearby major roads ($p < 0.1$); As, Ga, Mg, S & Se increase and Al, Ca, Ni & Si are not affected. In SW Denver, Ca, Mg & S peak when prevailing wind direction is 250 degrees (W/SW), suggesting a local emission source – possibly asphalt, concrete or brick manufacturing, gravel and sand mining, recyclable material wholesalers, or highway construction. Although none of the measured concentrations exceed regional EPA RSLs, epidemiological exposure assessment is needed to set accurate health guideline values for metal species in Colorado. Student support provided by T32GM144856.

Graduate Student/ERHS

69. Characterization of a cesium-137 gamma irradiator

Rebecca Mueller, Justin Bell, Maelle Coupannec, Ralf Sudowe

Due to the nature of radioactive decay, as a radioactive source ages, the activity of that radioisotope decreases. A 6000 Curie cesium-137 source was installed in room 470 of the Molecular and Radiological BioSciences Building in 1989. This work aims to characterize the dose rate that the source currently delivers to allow for its future use in experiments to study the effect of radiation damage on chromatographic materials. This is confounded by the fact that the half-life of cesium-137 is 30.05 years. Thus, more than half of the activity inclement to this source has decayed away. This work uses Fricke dosimetry as described in ASTM standard 1026-04 for the dose measurement. Samples are placed in batches of 8 in a rotating sample carousel which rotates at 0.2 Hz. Additive manufacturing was used to create the holders for the samples to best match planned irradiation geometries for further work. A dose response curve was constructed as the samples were irradiated over a variety of times (3 minutes to 18 minutes). The saturation point of Fricke dosimetry (400 Gy) was reached during longer irradiations (60 minutes). As the source was installed in the late 1980s, some details about it are difficult to determine from a proprietary point of view. Thus, the height of the source when deployed from the shielding was also triangulated and calculated during the course of this work. Lower irradiation times were used to determine the source's position as, at higher doses, the two layers of the sample carousel see more similar dose rates due to the scattering within the irradiation chamber. Research supported by the MAP ERC.

Graduate Student/ ERHS

70. Helping veterinary clients prepare for disasters for their pets: a CSU pilot study

Ashley L. Muller, Hanna D. Kiryluk, Danni Scott, Treana Mayer, Molly Carpenter, Colleen Duncan

Natural disasters are increasing in frequency and can negatively impact not only people, but also their pets. Previous research suggests that most pet owners do not have a current disaster plan but would be interested in receiving pet disaster preparedness information from their veterinary team. The objective of this project was to evaluate two means by which to communicate disaster preparedness, active versus passive, with pet owners during a veterinary appointment. The pilot study was conducted within the Community Practice (CP) section of CSU's Veterinary Teaching Hospital. A purpose-built website was created containing disaster planning resources, and a flyer was designed containing a QR code to the website. During the active engagement period, fourth-year veterinary students in CP were asked to physically hand the flyer to pet owners at appointment intake. During the passive engagement period, the same flyers were hung in CP exam rooms where pet owners would be able to see them. Both means of engagement were in place for two weeks. The frequency of interaction with the online content was generally low (5% of 178 pet owners); however, more clients scanned the QR code for the website during the active engagement period (8%) compared with the passive engagement period (2%). While this study was relatively small, it provides foundational information to inform future work on the development of effective strategies to communicate pet disaster preparedness information to pet owners.

DVM Student/MIP

71. A Study of Feline Immunodeficiency Virus Prevalence and Expert Opinions on Standards of Care

Mary Nehring, Ellyn M. Dickmann, Kara Billington, and Sue VandeWoude

The purpose of this study was to identify knowledge gaps regarding the global prevalence of Feline Immunodeficiency Virus (FIV). Additionally, we obtained professional opinions and experiences regarding FIV in selected countries. To accomplish these objectives, we reviewed ninety articles reporting on FIV prevalence between 1980-2017 and interviewed ten experts in feline medicine and retroviruses from different countries to determine regional perspectives. The results of this study showed that FIV prevalence generally fell within 5-8% with a global weighted prevalence of 4.7%. Experts estimated prevalence approximated literature review prevalence. Attitudes and recommendations for management were consistent among experts. The most reported clinical sign was dental disease. Experts noted that the disease progression is not well-defined and many FIV positive cats do not become ill. In conclusion, the global prevalence of FIV has not changed in over forty years. Experts agree that FIV is not typically a disease of high concern and is often associated with infections of the oral cavity. Recommendations for future research include analyses to determine co-pathogen and environmental factors that impact progression; assessment of lifespan impacts; investigations of treatment efficacy and side effects; mechanisms of FIV-associated oral disease and prevention strategies; and research regarding populations that might benefit from vaccination. Research supported by institutional funds.

Research Associate/ MIP

72. Canine and human osteosarcoma cell co-culture with primary lung fibroblasts modulates sensitivity to standard-of-care chemotherapy

Aaron D. Offermann, Eric P. Palmer, Kathryn E. Cronise, Lauren Alfino, Daniel P. Regan

A major focus of metastasis research over the last half century has been on tumor cell intrinsic determinants of therapeutic resistance. However, emerging data suggests that the collection of non-malignant host cells of distant metastatic sites, termed the tumor microenvironment (TME), may also extrinsically promote these processes. The lung is a top three metastatic site for all cancers, and preclinical data in mouse metastasis models of breast and other cancers suggests that resident lung fibroblasts (LF) are a key metastasis-promoting cell type of the lung microenvironment. Osteosarcoma (OS), the most common primary tumor of the bone, almost exclusively recurs in the form of lung metastasis in up to 30% of patients, despite aggressive adjuvant chemotherapy. While this organ tropism of OS proves significant biological relevance, very little is known regarding how OS cell interactions with resident cells in the lung promote this resistance to treatment options. This prompted the endeavor to characterize the effects of OS standard-of-care chemotherapy doxorubicin (DOX), methotrexate (MTX/Anthracycline), and Cisplatin (CIS/Platinum) in a dual-species *in vitro* OS cell survival/proliferation study. This involved primary human and canine donor-derived lung fibroblasts. Cell counts were executed via Incucyte live cell imaging of eight RFP-labeled cell lines (four per species) to generate dose-response curves and determine the Half-maximal Inhibitory Concentration (IC50) values for every cell line with each drug in mono- and LF co-culture. Quantitative analysis of cell proliferation, measured as the fold change relative to the negative control of each condition, demonstrated distinct modulation in OS cell survival/proliferation in OS LF co-culture when compared to OS monoculture. These data provide a foundation for future research aimed at determining the mechanism of LF mediated chemoresistance to develop rational combination therapies to resensitize OS cells to DOX, MTX, and CIS. Research supported by Boettcher Foundation Webb-Waring Biomedical Research Award; NIH R03OD028265.

Research Associate/MIP

73. Transcriptome Reference Map of Adult *Culex tarsalis* Ovaries

Hunter Ogg, Liz Mielke, Rebekah Kading, and Corey Campbell

Our research group is investigating the effects of Rift Valley fever virus (RVFV) on vector mosquitoes, e.g. *Culex tarsalis*, to better understand the molecular basis for viral maintenance in nature. While these species typically acquire RVFV through the blood of infected animals, they can pass RVFV to offspring through transovarial transmission, which could provide an epizootic reservoir between outbreaks. Toward better understanding of this process, we performed single-cell RNA sequencing (scRNA-Seq) on ovaries. Blood-fed *C. tarsalis* mosquitoes were held for 7 days until eggs were laid. Ovaries were harvested post-ovulation and RNA libraries prepared for Illumina single-cell sequencing using 3' technology. The resulting data was analyzed using Cellranger3, Seurat and SingleCellTK to identify cell types based on key transcriptomic markers. Sequencing data was correlated with a list of putative genes identified through BLAST, and these were compared with known marker genes in other Diptera. Ovarian cell transcriptomes mapped onto thirteen distinct clusters. Some prominent ovarian proteins with significant expression differences between cell types, such as oskar, CecA1, and VgR, are well characterized and suggest these findings are in-line with previous studies. These were used to classify hemocytes, germ stem cells, and adipocytes; our group is exploring putative markers for less studied cell types. This information acts as both a baseline for genetic expression in *C tarsalis* ovaries and lays groundwork for subsequent studies of differential expression of infected mosquitos. Our findings may eventually inform responses to a disease whose transmission and reservoirs are still poorly understood. We are also correlating this sequencing data with single-molecule inexpensive fluorescence *in-situ* hybridization (smiFISH) of *C. tarsalis* ovaries. We are illuminating marker genes alongside RVFV transcripts to provide a visualization of cell types and demonstrate the effects of viral infection on gene expression. Research support provided by USDA-NACA funding to C Campbell (Rosenberg).

PostBacc/ MIP

74. Unraveling Toll-like Receptor Dynamics in Attenuating Lung Inflammation from Organic Dust Exposure

Emmanuel Oyewole, Logan Dean, Alissa Threatt, Melea Barahona, Kaylee Jones, Maelis Wahl , Morgan Pauly & Tara M. Nordgren

Exposure to organic dust poses a substantial occupational health risk, often triggering pulmonary inflammation and potential long-term health implications. Within this context, specialized alveolar macrophages (AMs) assume a pivotal role in the initial response to inhaled particulate matter. Specifically, Toll-like receptors (TLRs) expressed by these AMs serve as key sensors for both microbial and damage-associated molecular patterns. This study aims to elucidate the intricate involvement of TLRs 2, 3, 4, and 9 in mitigating lung inflammation subsequent to organic dust exposure. Previous research conducted in our laboratory has indicated the participation of interleukin 22 (IL-22) in modulating inflammation. To investigate the dynamic roles of TLRs, we employed immunofluorescent staining techniques and monitored mean fluorescent intensity (MFI) at various time points (6hrs, 24hrs, 48hrs) following exposure to escalating concentrations of organic dust. Additionally, an inhibition assay is being designed to decipher the specific TLR(s) responsible for mediating pro-resolution. Our staining experiments revealed an intriguing pattern: for TLRs 2, 3, and 4, MFI increased in correlation with both dust concentration and time, indicating heightened TLR activation in response to organic dust exposure. However, TLR 9 displayed a contrasting trend, with decreasing MFI over time, suggesting a distinctive regulatory role. Understanding the precise contributions of TLRs in the context of organic dust exposure and their impact on lung inflammation is paramount. The findings imply that TLRs 2, 3, and 4 play substantial roles in the initial response, whereas TLR 9 may possess distinct regulatory functions. Ongoing and forthcoming assays aim to offer a more comprehensive understanding of AM involvement in resolving lung inflammation, potentially unveiling novel avenues for therapeutic interventions to mitigate the health risks associated with organic dust exposure. Research support provided by NIH/NHLBI R01HL185926.

Graduate Student/ERHS

75. Transcriptomic responses in synovial fluid cells and circulating leukocytes in horses with progressive osteoarthritis

Ashana Patel, Gabriella Piquini, Lyndah Chow, Renata Impastato, Dean Hendrickson, Steve Dow, and Lynn Pezzanite

Given the protracted asymptomatic pre-radiographic phase of OA in many cases, improved understanding of the early changes occurring both within the joint and in circulating leukocytes could improve our ability to diagnose the disease and tailor treatments. The objective of this study was to investigate the transcriptomes of synovial fluid cells and circulating leukocytes in horses with and without progressive OA, using RNA sequencing. This study will address the overall hypothesis that horses with OA will have a unique transcriptome response in both blood and synovial fluid that can be identified using recently available next-generation sequencing techniques. Blood and synovial fluid were collected from 6 horses with OA presenting for arthroscopy and 12 healthy horses without OA. Horses were determined to either have OA or be disease free via musculoskeletal palpation, lameness evaluation and radiographic examination. RNA was extracted from synovial fluid cells and peripheral blood mononuclear cells and subjected to full RNA sequencing using an Illumina-based platform. Our early results indicate that synovial fluid cell transcriptomes are significantly different in horses with OA, and importantly the circulating leukocyte responses are also significantly different. Thus, the use of next gen sequencing tools can provide unique new insights into the pathogenesis of OA in horses, and suggest important new diagnostic tools for non-invasive diagnosis and monitoring of disease activity. Student support was provided by the National institute of Food & Agriculture at the United States Department of Agriculture : USDA-NIFA-AHDR-COLV-23

DVM Student/ CS

76. Transcriptomic response of equine synovial tissues following immune conditioned cellular therapy to treat septic arthritis

Cody Plaisance, Lyndah Chow, Julie Engiles, Laurie Goodrich, Kelly Santangelo, Steven Dow, Lynn Pezzanite

Multidrug antimicrobial resistance represents an emerging global 'One Health' issue in human and veterinary medicine. Development of antimicrobial resistance has prompted investigation of adjunctive therapeutic strategies to reduce antimicrobial usage, including regenerative therapies such as mesenchymal stromal cell (MSC)-based treatments. Previous work from this group have demonstrated that MSC antimicrobial properties can be enhanced through conditioning with Toll-like receptor (TLR) and Nod-like receptor ligands prior to in vivo administration. Using an equine preclinical model of septic arthritis, we demonstrated that treatment of septic joints with MSC activated with the TLR-3 agonist polyinosinic:polycytidylic acid (pIC) plus vancomycin antibiotics (TLR-MSC-VAN) reduced pain scores, quantitative bacterial counts in synovial fluid (SF) and synovium, and pro-inflammatory cytokines IL-18 and IL-6 in SF vs. vancomycin alone (VAN). In the present study, we investigated this observed clinical improvement with TLR-MSC therapy mechanistically, leveraging recently available Next-Generation Sequencing (NGS) techniques. Synovial tissues were collected from 8 horses with Staphylococcal septic arthritis of the tibiotarsal joint treated intra-articularly with either TLR-MSC-VAN or VAN alone. Tissues were fixed in neutral-buffered 10% formalin and zinc fixative. Formalin-fixed paraffin-embedded (FFPE) synovial tissues from dorsolateral joint quadrants were sequenced using a custom-designed 200-gene equine Nanostring nCounter immune panel via sequence-specific mRNA probes to directly detect transcript expression levels. Comparison of transcriptomes in synovium of TLR-MSC-VAN vs VAN treated horses revealed moderate changes in gene expression, with upregulation of 9 and downregulation of 17 genes (based on fold change ≥2 or ≤-2 and significant FDR (false discovery rate) adjusted p-value of ≤0.05). Upregulated genes in TLR-MSC-VAN treated horses included those related to T-cell recruitment. summary, transcriptomic analyses using a novel equine Nanostring immune panel provided new and previously unpublished insights into how innate and adaptive immune cells within synovial tissues respond to TLR-activated MSC treatment when used to treat orthopedic infection. Research support provided by Grayson Jockey Club Research Foundation, ACVS Zoetis Dual Training Grant, NIH/NCATS CTSA TL1TR002533, NIH T32ODO010437, Verdad Foundation, Charles Shipley Family Foundation and Carolyn Quan and Porter Bennett. Student support provided by NIH PREP R25GM148297.

PostBacc/CS

77. Characterizing the FAT-1 mouse strain in a non-surgical model of ACL rupture

Ashley M. Potter, Emily M. Van Zealand, Jeremiah T. Easley, Tara M. Nordgren, Katie J. Sikes

Anterior cruciate ligament (ACL) injuries are very common. Acutely, ACL injury activates the inflammatory cascade; when left unresolved this leads to chronic inflammation and a higher risk for the development of post-traumatic osteoarthritis. Omega-3-fatty acids (ω-3 FA) are suggested to have anti-inflammatory effects; however, the mechanisms by which they could attenuate inflammation following ACL rupture are not well understood. Our overall goal is to characterize the role of ω-3 FA on inflammation and structural and functional knee joint changes following ACL rupture. Using FAT-1 transgenic mice that possess elevated levels of ω-3 FA, the aim of these pilot studies was to ensure that FAT-1 mice are comparable to wild type (WT) mice in their susceptibility to ACL rupture in a mechanical model. Under IACUC approval, unilateral ACL rupture was achieved via mechanical tibial displacement in male and female WT and FAT-1 mice at 15-17 weeks old (n=5/genotype/sex). Weights, tibial lengths and loading curve outputs were compared using ANOVA for sex, genotype, and sex/genotype interaction factors. No significant differences were observed between FAT-1 and WT mice with respect to weight (p=0.5518), tibial length (p=0.1857) or peak tibial displacement force (p=0.9601), and 100% of mice demonstrated a full, midsubstance ACL rupture. The lack of significant differences observed between FAT-1 and WT mice show that there are no appreciable differences in the FAT-1's eligibility to undergo ACL rupture via mechanical tibial displacement as previously shown with WT mice. FAT-1 mice are thus an eligible model to observe the effects of ω-3 FA on inflammation response post ACL rupture. Future work will include genomic, proteomic, functional, and structural assessments in both genotypes following ACL rupture, to ascertain potential beneficial effects of ω-3 FA during recovery following ACL injury.

Graduate Student/ ERHS

78. Preparing Pets and their People

Joedy Quintana, Valeria Sanchez, Lindsey Viola, Danielle Scott, and Colleen Duncan

In December 2021 over 1,000 pets died in their homes when the Marshall wildfire spread in Boulder County (1). Climate change has made disasters, and the associated health risk, more frequent and severe. Despite this growing risk, a 2022 study revealed that 55% of adults in the US do not have a disaster plan (2). For those who have disaster plans, it remains unknown how many of these plans include pets. The objective of this project was to explore the potential for veterinary teams to facilitate the development of a pet-inclusive disaster plan by discussing the topic at routine veterinary visits. We hypothesized that the topic of disaster preparedness was not typically covered during veterinary visits, but that pet owners would be receptive to learning about the topic from their veterinary team. We conducted two separate anonymous surveys, one for veterinarians and one for veterinary clients in the US. Veterinary teams were asked how often disaster plans are discussed with veterinary clients and if their clinic had a disaster plan in place. Pet owners were asked if they have disaster plans that include their pets, if their veterinary team ever discussed the topic and if they would be interested in learning more about disaster plans involving their pet. Data collection is underway; the first survey is currently distributed through veterinary networks (social media, emails, conferences). The second survey is distributed through Amazon Mechanical Turk. Results of this project will serve as a foundation upon which to promote disaster planning at veterinary clinics. Such efforts could potentially increase the number of animal lives saved during a natural disaster. Student support provided by Boehringer Ingelheim Veterinary Scholar Program.

DVM Student/MIP

79. *Anopeheles stephensi* mosquitoes: interrogating their metabolic profile and cryopreservation methods.

Gabriela Ramirez, Emily N. Gallichotte, Ashley Freedman, Michael C. Young, MaKala Herndon, Elisha Xiao-Kim, Jackson Watkins, Madison Stolz, Maria Alexandra Marquez, Gaukhar Iskakova, Jennifer Barfield, Gregory D. Ebel, Karen Dobos

Aedes, Culex, and *Anopheles* mosquitoes are the vectors for pathogenic viruses such as Zika, dengue, chikungunya, as well as the *Plasmodium* parasite that causes malaria. At present, many key mosquito colonies must be maintained year-round which may lead to the loss of engineered genetic markers and traits, laboratory adaptation, and population bottle necks that may impact genetic and phenotypic traits of the colonized mosquitoes. Development of methods to cryopreserve mosquitoes could help mitigate some of these issues. We study cryopreservation of *Anopheles stephensi* (*An. stephensi*) mosquitoes due to their high vectorial competence for *Plasmodium* and their geographical distribution has recently expanded. We hypothesize that *An. stephensi* mosquitoes have unique metabolic markers for each life stage, especially at the first and second larval stages (L1 and L2) and that these markers may point toward targets that can be exploited to increase recovery rate after cryopreservation. We generated metabolic profiles of *An. stephensi* mosquitoes at each life stage. We found distinct metabolic profiles between all life stages, including L1 and L2, in spite of minor anatomical and phenotypical changes. We demonstrated that we could successfully super cool L1 at -15°C for 10 minutes with recovery and subsequent fecundity through multiple additional generations. With this data in hand, our future studies will explore whether discreet metabolic markers of L1 versus L2 predict survivability during and after super cooling. Additionally, it can be used to further advance the development of cryopreservation processes for *An. stephensi* and expand efforts of mosquito cryopreservation efforts at large.

Graduate Student/ MIP

80. Translational inhibition of NLRP3 and NF-κB is protective in Experimental Autoimmune Encephalomyelitis (EAE) Mouse Model of Multiple Sclerosis

Sydney Risen, Grace Weisman, Sadhana Sharma, Prashant Nagpal, Anushree Chatterjee, Vincenzo Gilberto, and Julie Moreno

Multiple sclerosis (MS), an autoimmune-mediated disorder that affects the central nervous system, is one of the most prevalent neurodegenerative disabilities. Inflammation due to immune cell infiltration and oligodendrocyte death are the primary causes of CNS plaques, demyelinated and transected axons, and astrogliosis in both white and gray matter. These lesions inhibit the correct transmission of nerve impulses and lead to neuronal dysfunction. Currently, there are little to no effective treatments to halt or slow the progression of MS. TNF-α, NF-κB, and NLRP3 are key inflammatory signaling molecules known to be upregulated in MS and other neurodegenerative diseases alike. Sachi Bioworks™ developed two therapeutics that translationally inhibit both TNF-α and NF-κB (SB_NI_111) or NF-κB and NLRP3 (SB_NI_112). Established by PK/PD studies, these therapeutics are systemic but nontoxic to mice, at doses up to 500mg/kg. We hypothesized that by treating Experimental Autoimmune Encephalomyelitis (EAE) induced mice with the neurotherapeutics, glial inflammation could be decreased, demyelination halted, neuronal loss slowed, clinical progression prevented, and lifespan expanded. Mice inoculated with EAE were intraperitoneally injected with SB_NI_111, SB_NI_112, or vehicle 3x per week at 150 mg/kg. Mice were monitored daily for clinical progression of disease state. We found translational inhibition of NF-κB and NLRP3 prevented clinical signs of EAE, significantly reduced activation of microglia and infiltration of macrophage into the spinal cord, and protected against demyelination within the spinal cord of mice inoculated with EAE. SB_NI_111, inhibition of TNF-α and NF-κB, showed far less effective than that of SB_NI_112, indicating NLRP3, mores than TNF-α, is a key pathway in the progression of EAE and MS. Current research efforts are being completed with oral dosing measures of SB_NI_112. Furthermore, these therapeutics have shown promise in murine models of Prion Disease, Alzheimer's Disease, Parkinson's Disease, and Aging. Research supported by NASA SBIR 80NSSC22CA116, Sachi Bio, and CVMBS Murphy Turney Fund at Colorado State University.

Graduate Student/ERHS

81. The autism-associated loss of δ-catenin functions disrupts social behavior

Regan L. Roach, Hadassah Mendez-Vazquez, Rahmi Lee, Scott Roh, Matheus Sathler, Madeleine C. Moseley, Rosaline A. Danzman, Jessica P. Roberts, Libby Koch, Seonil Kim

Social behavior is essential to survive for many species, and various mental disorders have social impairment as a primary symptom. Research suggests that synaptic activity and signaling can regulate social behavior. However, the links between synaptic regulation and social behavior are not completely understood. δ-catenin functions as an anchor for the glutamatergic AMPA receptor (AMPARs) to regulate synaptic activity in excitatory synapses. Mutations in the δ-catenin gene are found in autism patients from multiple families and induce a loss of δ-catenin functions at excitatory synapses, which is thought to be the etiology of autism in people. Recent studies, including our own, suggest that the loss of δ-catenin functions induced by δ-catenin knockout (KO), the autism-associated δ-catenin missense mutation, or shRNA-mediated δ-catenin knockdown significantly decreases cortical neurons' inhibition to increase excitation. Our new data further reveal that gamma powers in the medial prefrontal cortex (mPFC) are significantly reduced in δ-catenin KO mice. We also identify that the loss of δ-catenin functions significantly disrupts social behavior in mice. This suggests that δ-catenin deficiency impairs prefrontal neural activity at cellular and network levels to induce social deficits. Research supported by NIMH, The Jerome Lejune Foundation, and CVMBS.

Graduate Student/ BMS

82. Current Antimicrobial Use in Horses Undergoing Exploratory Celiotomy: A Survey of Board-Certified Equine Specialists

Meagan Rockow, Gregg Griffenhagen, Gabriele Landolt, Dean Hendrickson, Lynn Pezzanite

In the past decade, there has been a considerable increase in the recognition of antimicrobial resistance in equine practice. The objective of this study was to survey the current clinical use of antimicrobials for a commonly performed surgical procedure (exploratory celiotomy) with the goal of understanding how recent literature and changes in microbial resistance patterns may have impacted antimicrobial selection practices. An electronic survey was distributed to veterinary professionals within the American College of Veterinary Internal Medicine (ACVIM) and the American College of Veterinary Surgery (ACVS). A total of 113 completed surveys were returned. Practitioners reported antimicrobials were most frequently given 30-60 min preoperatively (63.1%). Two antimicrobial classes were typically administered (95.5%), with gentamicin (98.2%) and potassium penicillin (74.3%) being the most common. Antimicrobials were typically not re-dosed intraoperatively (78.6%). Factors that affected overall treatment length postoperatively included resection (81.4%), bloodwork (75.2%), enterotomy (74.3%), fever (85.0%), incisional complications (76.1%), and thrombophlebitis (67.3%). The most common duration of antimicrobial use was 1-3 d for non-strangulating lesions (54.4% of cases) and inflammatory conditions such as enteritis or peritonitis (50.4%), and 3-5 d for strangulating lesions (63.7%). Peri-incisional and intra-abdominal antimicrobials were used by 24.8% and 11.5% of respondents, respectively. In summary, antimicrobial usage patterns were highly variable among practitioners and, at times, not concordant with current literature. Research supported by a Young Investigator's grant from the Center for Companion Animals at Colorado State University.

DVM Student/CS

83. Transcriptomic Insights into Advanced Maternal Aged Granulosa Cells Emphasizes Inflammation and Immune Response

Heather Rogers, Ahmed Gad, Gentry Cork, Nico Menjivar, Melinda Meyers, Dawit Tesfaye, and Ye Yuan

Women are delaying motherhood, leading some to seek fertility treatments; however, advanced maternal age (AMA) fertility patients (>38 years old) experience decreased success rates. The decreased success can be attributed, in part, to quantity and quality of oocytes, which is significantly influenced by surrounding granulosa cells. In this study, we compared mRNA and miRNA transcriptomes between young (<32 years old) and AMA patients' granulosa cells to identify ovarian aging molecular signatures. Granulosa cells were isolated from 30 patients' follicular fluid (15 in each group), and RNA was isolated using miRNeasy Micro Kit according to the manufacturer's instructions. RNA samples from each patient were pooled into groups of three limiting individual variation (n=5 each). Samples were sent to NovoGene for library preparation and sequencing. The Illumina NovaSeq platform was utilized for paired-end 150 base-pair sequencing of mRNA and single-end 50 base-pair sequencing of miRNA. Differential miRNA and gene expression analyses and conventional cluster analyses were performed on QIAGEN CLC Genomics Workbench software. Gene ontology and pathway analysis were performed using the Ingenuity Pathway Analysis software. We identified 293 and 21 differentially expressed genes (DEGs) and miRNAs, respectively, between young and AMA granulosa cells (P value < 0.05, FDR < 0.25, Fold Change > 1.5). Pathway analysis indicates DEGs play a role in inflammation, immune response, cytokine signaling, and cellular processes. To further investigate the regulatory mechanisms, we looked at the convergence of DEGs with predicted target genes of differentially expressed miRNA. Pathway analysis of this intersection indicates a regulation of pathways involved in immune response, inflammation, fibrosis, and cellular signaling. This research can aid in identifying specific genes, miRNAs, or pathways, which could be therapeutic targets to mitigate the impacts of aging, like inflammation, for AMA fertility patients. Support for RNA sequencing provided by the Colorado Center for Reproductive Medicine.

Graduate Student/ BMS

84. Prenatal exposure to valproic acid reduces synaptic δ-catenin levels and disrupts ultrasonic vocalization in neonates

Scott Roh, Hadassah Mendez-Vazquez, Matheus F. Sathler, Michael J. Doolittle, Anastasiya Zaytseva, Hannah Brown, Morgan Sainsbury, Seonil Kim

Valproic acid (VPA) is an effective and commonly prescribed drug for epilepsy and bipolar disorder. However, children born from mothers treated with VPA during pregnancy exhibit an increased incidence of autism spectrum disorder (ASD). Although VPA may impair brain development at the cellular level, the mechanism of VPA-induced ASD has not completely addressed. The previous study has found that VPA treatment strongly reduces δ-catenin mRNA levels in cultured human neurons. δ-catenin is important for the control of glutamatergic synapses and strongly associated with ASD. VPA inhibits dendritic morphogenesis in developing neurons, which is also found in neurons lacking δ-catenin expression. We thus hypothesize that VPA prenatal exposure significantly reduces δ-catenin levels in the brain, which disrupts glutamatergic synapses to cause ASD. Here, we found that VPA prenatal exposure markedly reduced δ-catenin levels in the brain of mouse pups. VPA exposure also impaired the dendritic development in developing mouse cortical neurons, which was reversed by elevating δ-catenin expression. VPA treatment significantly impaired ultrasonic vocalization (USV) in newly born pups when they were isolated from the nest. We also found that VPA exposure was unable to activate neurons in the hypothalamus, which is required to produce animals' USVs following isolation from the nest. Finally, VPA exposure significantly reduced synaptic AMPA receptor levels and postsynaptic density 95 (PSD-95) in mouse pups, which likely contributed to deficits in neuronal activation in the hypothalamus. Therefore, these results suggest that VPA-induced ASD pathology can be mediated by loss of δ-catenin functions. Research support provided by the NIMH, The Jerome Lejune Foundation, and CVMBS.

Graduate Student/BMS

85. Air quality at United States' equine eventing competitions

Valeria Sanchez, Danni Scott, Joedy Quintana, Lindsey Viola, Katie Seabaugh, Erin Contino, Sheryl Magzamen, Colleen Duncan

The health impacts of air pollution are extensive, well documented and transcend species. Athletes are of particular concern given their higher effective dose secondary to increased ventilation rates, a factor that poses particular risks to horses due to their high tidal volume. Previous work at Colorado State University has found that air pollution significantly impacts competition speed of racehorses, but little is known about impacts in other equestrian sports. The objective of this project was to characterize the air quality at United States equine eventing competitions. We obtained a list of events with competition dates and locations from the United States Eventing Association (USEA) for the period of January 1, 2008, to December 31, 2022, and merged it with Environmental Protection Agency's daily air quality data at the county level (daily air quality index (AQI) values, AQI category, and defining parameter e.g., Ozone, Particulate matter 2.5). There were 2147 events over 5483 days (15 years) spanning 10 USEA geographic regions. There were statistically significant differences in the mean daily average AQI by year, month and USEA region. A statistically significant observation was made regarding the frequency of competition days and events occurring when the air quality deviated from the 'good' category (AQI > 50) or exceeded the threshold (AQI > 150) at which the United States Equestrian Federation 'strongly recommends' cancellation of competitions. Of interest were regions 6 and 9, that most frequently held competitions during elevated AQI periods. While rare (n=26, 0.5% of total competition days), the frequency of competition during periods of unhealthy air quality was significantly more common in the most recent third (5 years) of the study period. Collectively, this information empowers horse owners and equine sport governing bodies to elevate communication and refine event planning, placing a paramount focus on the health of the horses.

DVM Student/ MIP

86. Investigating the origin and nature of half-crossover cascades in *Saccharomyces cerevisiae*

Camryn Schmelzer, Victoria Harcy, Brissa Santacruz, Sean Merriman, Ruth Watson, Juan Lucas Argueso

A half-crossover (HC) cascade is a mutagenic sub-pathway of homologous recombination (HR), where one DNA double-stranded break (DSB) is able to trigger multiple chromosomal translocations. When a DSB is formed but only one of the two broken ends is captured to engage in HR, the repair will proceed through Break-Induced Replication (BIR). In BIR, the single-ended DSB invades a homologous donor sequence and uses it to initiate DNA replication that extends through the end of the template chromosome. Failure of BIR initiation and/or irregular processing of BIR intermediates can lead to HC formation, in which the single-ended DSB is healed through a translocation, breaking the donor molecule in the process, leaving it with only one DNA end to engage in HR repair. This futile repair attempt may spawn another half-crossover, thus resulting the "cascading" nature of the mechanism. HC cascades can lead to cycles of translocations until BIR occurs to end the series of rearrangements. Structure Selective Endonucleases (SSEs) have been implicated in HC formation. Generally, SSEs cut DNA joint molecules to conclude HR repair. However, the premature activation of SSEs, such as Mus81 and Yen1, during BIR may generate HCs. Additionally, Pol32 is an accessory subunit of Polδ and largely required for BIR; cells deficient for Pol32 are likely predisposed to HC cascade formation. We have constructed an experimental system in *Saccharomyces cerevisiae* designed to phenotypically identify clones that specifically experienced a cascade of HCs followed by one BIR event that heals the chromosome. We are investigating the roles of Mus81, Yen1, and Pol32 in HC cascade formation, measuring the frequency of HC cascades, and characterizing the resultant rearrangements through Pulse Field Gel Electrophoresis (PFGE) and targeted long-read sequencing across translocation junctions. We postulate HC cascades result from an imbalance between BIR initiation and SSE activation. Research supported by NIH MIRA R35 award.

PostBacc/ERHS

87. Uncovering biomarkers to improve identification of latent tuberculosis infection and prediction of disease outcome

Kimberly Shelton, Luisa Maria Nieto Ramirez, Marisa Harton, John Belisle, Mark Hatherill, Karen Dobos

One-quarter of the world's population is estimated to be infected with *Mycobacterium tuberculosis* (*Mtb*), which causes tuberculosis (TB). TB can present on a spectrum from active disease (in which people are symptomatic and infectious) to latent disease (non-symptomatic and non-infectious). Latent TB can be challenging to reliably detect, and it is not possible to predict progression from latent to active disease, posing an obstacle to reducing TB burden. Improved diagnostic tools that can reliably detect TB across disease stages, particularly latent TB, along with predict risk of progression to active disease are needed. To address this, we are investigating potential biomarkers, in the form of *Mtb* proteins in host exosomes. Exosomes are a type of extracellular vesicle shown to have a role in many diseases. We hypothesize that we can detect *Mtb* proteins in exosomes isolated from serum samples of patients with latent TB, using mass spectrometry (MS), and that we can identify an association between these proteins, disease stage, and outcome following latent TB. Our current project aims to validate a set of potential biomarkers, in the form of *Mtb* peptides in host exosomes, which were previously detected in a targeted MS assay, on a larger cohort of samples across disease stages. To this end, we performed three exosome isolation methods on a set of serum samples from our current sample cohort and characterized the resulting exosomes through Nanoparticle Tracking Analysis (NTA), western blot, and mass spectrometry. From these results, we selected the optimal exosome isolation method and applied this method to the current sample cohort. We have also developed a Parallel Reaction Monitoring-MS (PRM-MS) method which detects the set of *Mtb* peptides previously identified, and we are applying this to our current sample set, which will enable us to identify the profile of bacterial peptides across disease stages. Project support by RePORT – Regional Prospective Observational Research for Tuberculosis in the Republic of South Africa, CRDF Global, Project Number R-202109-68100, PI Dr. John Belisle; Trainee support by Infectious Disease Research and Response Network Training Program T32AI162691, PI Dr. Gregg Dean.

Graduate Student/ MIP

88. Validation of Cannabigerovarinic acid (CBGVA) Pharmacokinetics in C57BL6 mice.

Payton Shirley, Celine Campos, Gregory Dooley, Stephanie McGrath, and Julie A Moreno

Epilepsy affects 3.4 million individuals nationwide and approximately 75% of the canine population. Hallmarks of epilepsy include hyperexcitability of neurons and hypersynchrony of neuronal networks, resulting either in irregularity or impairment in cells responsible for inhibiting excitatory cells decreased electrical signals. Alternatively, it may result from an excess production of neurochemicals triggering abnormal discharge of electrical signals, or a combination of both factors. Cannabigerovarinic acid (CBGVA) is a precursor molecule of Cannabidiol (CBD), a previously approved anti-convulsant drug for epilepsy. Minimum research has been conducted on CBGVA however, it may be a promising anti-convulsant drug for epilepsy. We hypothesize that by utilizing Cannabigerovarinic acid (CBGVA) in C57BL6 mice we can downregulate three key epileptic targets to display anti-convulsant properties. However, prior to this we must determine pharmacokinetics and pharmacodynamics for the compound. We have currently conducted a method of validation for CBGVA using Liquid Chromatography Mass Spectrometry (LC-MS). This validation and optimization of the method will allow us to determine the limit of quantification, accuracy, precision, recovery, stability, and interference of CBGVA within mouse plasma and brain homogenates. Using LC-MS as a suitable analytic method to support the pharmacokinetic (PK) studies regarding CBGVA is critical to the future of the project. Future studies will utilize these PK results to better understand the mechanism of action of CBGVA on activation of T-type calcium channels within the brain that allow for inhibition of epilepsy in both humans and companion animals.

Graduate Student/ERHS

89. Development and validation of a new coma scale and long-term outcome scoring system for dogs with Traumatic Brain Injury (TBI)

Britta Siegenthaler, Claire Tucker, Kelly Hall, Tracy Webb, Ayla Mollen, and Katy Waldrond

Head injury and associated traumatic brain injury (TBI) is a common cause of emergency room presentation in both dogs and humans. The current coma scale for dogs, the Modified Glasgow Coma Scale, is not specific for TBI and patients with spinal cord injuries may be incorrectly diagnosed as having TBI. We developed a new coma scale, the veterinary Glasgow Coma Scale (V-GCS), that removes the focus of spinal cord reflexes in the motor component of the assessment. We hypothesize that this new scale will be more specific for traumatic brain injuries and can accurately predict patient outcomes. Additionally, there is a significant need for a long-term outcome scoring system for dogs so that canine TBI can be utilized as a translational model in future clinical trial studies for novel therapies. We have designed a modified version of the Glasgow Outcome Scale – Extended Pediatric version (GOS-EP), called the Veterinary Glasgow Outcome Scale (V-GOS), that will be scored in patients at two weeks, one month, and six-month post-head injury. We hypothesize that this scoring system will correlate with the coma scale scores measured at the time of presentation and will be sensitive to severity of injury. Student support provided by Boehringer Ingelheim Veterinary Scholars Program.

DVM Student/ CS

90. Pilot: An approach to assessing enrichment items as modifiers of daily activity in laboratory guinea pigs

Benjamin Singh, Alexa Spittler, Marion Desmarchelier, Lon V. Kendall, Kelly S. Santangelo, Miranda J. Sadar

Environmental enrichment has become a standard aspect of modern laboratory animal care in both the United States and Europe. The benefits of enrichment have been well documented, including promotion of species-specific behaviors, reductions in stereotypies, reduced levels of anxiety and alterations in stress physiology, and key roles in brain function. A pilot study was performed using guinea pigs (n=3, female) to assess two factors to aid in the design of a larger project: (1) the environment (open-field enclosure versus home cage) that animals were most likely to interact with enrichment items; and (2) initial preference for items so those of more versus less interest could be prioritized. For the environmental preference stage, animals were acclimated to an open-field enclosure (4'x4'x2.3') over two weeks. Animals were then exposed to 3 pre-selected enrichment items (one food, one interaction, one foraging) and their standard enrichment item (red hut) for 30 minutes over three days to determine interactions with items. Activity/behavior was assessed using a species-specific ethogram. In the open-field, guinea pigs spent the majority of their time either exploring the enclosure or sleeping in the hut. After one week, exposure to the items and hut was repeated in each animal's home-cage environment. In the home-cage, guinea pigs demonstrated a greater interest in interacting with enrichment items. Armed with this information, investigation can move forward with determining whether exposure to these preferred enrichment items will lead to positive alterations in the daily activity budget of each species, with a particular focus on sex comparisons. Research support provided by Oxbow.

Resident/CS

91. Determination of strontium-90 and cesium-137 in freshwater fish near the Fukushima Daiichi Nuclear Power Plant

William Stephenson, Naofumi Akata, Donovan Anderson, Hirofumi Tazoe, Ralf Sudowe

In the wake of the Fukushima Nuclear Power Plant (FNPP) accident in Japan, commercially sold food products from the Fukushima prefecture have come under increased scrutiny by the public due to potential bioconcentration of radionuclides. The fish that are harvested from fresh and salt waters are one such product of interest. The main isotopes of concern in fish are strontium-90 (^{90}Sr) and cesium-137 (^{137}Cs). These are radionuclides that have been found to substitute stable calcium and potassium in bone and muscle tissue, respectively. This research will explore the concentration of ^{90}Sr and ^{137}Cs in freshwater fish sampled near the FNPP to determine if they are safe for consumption. This assessment is important in assuaging the fears of the public regarding the safety of products exported from the Fukushima region, allowing for commerce to flourish once again in the Fukushima prefecture. Fish have been sampled from the Ukedo River located northwest of the FNPP. Tissue and bone samples were separated for the fish and dried. Sample aliquots were divided between Colorado State University and Hirosaki University for a comparison study using the same dissolution and separation method. Tissue samples were dissolved in 8 M HNO3 and measured via gamma spectrometry on a HPGe to determine Cs-137. Bone samples were ashed and dissolved in 8 M HNO3 for separation of ^{90}Sr and yttrium-90 (^{90}Y) via Eichrom DGA resin. Analysis and determination of ^{90}Sr was completed via liquid scintillation counting at Colorado State University and via inductively coupled plasma mass spectrometer at Hirosaki University. Sample results and method limits of detection will be compared to those obtained by our collaborators at Hirosaki University. In the fish samples, ^{137}Cs was determined to be present in one composite sample, with activity of 3904 ± 160 Bq/kg dry weight, near the Ogurasawa Bridge. Research supported by the FDA.

Graduate Student/ ERHS

92. A novel co-culture model highlights extrinsic modulation of TNBC therapy responses by primary lung fibroblasts

Claire Stratton, Marika Klosowski, Daniel Regan

Triple negative breast cancer (TNBC) has a poor prognosis due to high metastatic incidence, lack of molecular drug targets, and chemoresistance. Cancer associated fibroblasts extrinsically drive chemoresistance in primary TNBC tumors, but it is unclear if resident fibroblasts of distant metastatic tissues, such as the lung, influence TNBC drug sensitivity. Thus, we developed a breast cancer cell (BCC) - donor-derived lung fibroblast (LF) co-culture model, and hypothesized that anti-cancer drug screening using the TNBC cell line MDA-MB-231 in this model would uncover mechanisms of LF-mediated chemoresistance and therapeutic vulnerabilities relevant to lung metastasis. A high-throughput screen of 900 kinase inhibitor compounds revealed that while MDA-MB-231 drug resistance broadly increased in co-culture compared with monoculture, co-culture sensitivity increased to only three drugs, of which two were vacuolar protein sorting 34 inhibitors (VPS34i). Validation of prioritized compounds across a dose range confirmed enhanced co-culture resistance to MCB-613, a steroid receptor coactivator stimulator, and sensitivity to VPS34i drugs. As VPS34 is a key inducer of autophagy, we sought to determine if autophagy is a mechanism for MDA-MB-231 LF-mediated chemoresistance. Indeed, combined therapy with a VPS34i reversed LF-mediated resistance to TNBC standard-of-care doxorubicin. Ongoing experiments will compare autophagic flux between mono- and co-culture and determine if VPS34i therapy also reverses resistance to MCB-613, whose metabolic effects are known to be mitigated by autophagy. Together, these data support BCC-LF co-culture as a relevant in vitro model of lung metastatic TNBC that can inform preclinical drug development. Research supported by NIH K01OD022982. Student support provided by NIH training grant T35OD015130.

DVM Student/MIP

93. Sustainability at the CSU veterinary teaching hospital: identifying areas of improvement to inform future action items

Caroline Sullivan, Erik Gary, Arielle Gold, Claire Hood, Lindsey Viola, Danni Scott, Colleen Duncan

The purpose of this project is to determine veterinary staff perceptions of sustainability and potential departmental action items to decrease the CSU Veterinary Teaching Hospital's environmental footprint. In-person interviews using a standardized set of questions were conducted with 5 specialties of interest: Critical Care Unit, Internal Medicine, Exotics, Oncology, and Orthopedic Medicine and Mobility. Responses were then analyzed across all departments for similarities. Participating staff were asked to self-rate their department's sustainability efforts. Additionally, each specialty was asked to list specific sustainability issues relevant to their unique area of veterinary medicine. The average sustainability self-rating across all units was 2.4 (1 = Poor, 5 = Excellent). All units named both general and specialty-specific concerns. The common concerns shared amongst all the interviewed specialties included lack of proper recycling education, single-use plastics (specifically syringe casings and thermometer covers), disposable surgical gowns, food waste, and client disposal of medications. Among potential areas of improvement, several departments requested upgrades to facility bike racks, implementation of telemedicine appointments when possible, and digitizing medical records to reduce paper use. The project's results highlight that sustainability is nuanced. Waste was a common issue brought up, yet it accounts for only a small fraction of the VTH's emissions. The results of this study reflect a common interest amongst veterinary professionals to improve sustainable practices within the field but demonstrate a need for improved education to support behavioral changes that will have a measurable impact.

DVM/MPH Student/ MIP

94. The benefits of mentoring underserved high schoolers in veterinary medicine and One Health through a free, experiential college course

Gwen R. Svenson, Olivia R. Arnold, Patricia Vigil, Naomi Nishi, Christianne Magee

The field of veterinary medicine is predominantly composed of white professionals. A lack of mentorship and a significant financial burden are just a few of the barriers that continue to widen the gap for those aspiring to join the profession. To address these obstacles, Colorado State University and the Alliance Partnership have joined with Zoetis to devise the Veterinary Perspective Institute (VPI), a free, one-credit course that aims to provide minoritized and marginalized youth with critical mentoring for relevant veterinary experience and offers positive mentor relationships and strategies for overcoming obstacles. Launched with virtual enrollment in 2021 (n=23), this program has since been offered as a residential summer course in 2022 (n=35) and 2023 (n=17). Mentored by undergraduate, graduate, and veterinary students (n=10/year) and using a One Health approach, veterinary medicine is deconstructed, and misconceptions about the veterinary profession are unveiled while the desire to help others becomes central to career interests. Students are presented with case studies, research activities, hands-on experiences, and guest speakers that provide new perspectives on pursuing a career in helping others. College readiness material emphasizes the power of a growth mindset by encouraging the students to consider their goals, self-care, and the importance of mental health as they navigate their future aspirations. As a result of this program, all participating high school students have an improved understanding of veterinary medicine, an increased preparedness to attend college, and the personal steps needed to pursue their career goals in a healthy and feasible manner. Colorado State University is a predominantly white institution, and this program also creates opportunities for students, faculty, and staff to develop awareness, knowledge, and skills for working with diverse populations. Thanks to the funding provided by Zoetis and Dr. Christianne Magee's efforts as the principal investigator, the VPI program has been launched successfully for 3 consecutive years.

Staff/BMS

95. Parvovirus antibody titers in sera from dogs in a community based blood donor program

Kristine Kofron, Charles T. Talbot, Melissa Brewer, Rae Isdale, Sarah B. Shropshire, Kristin M. Zersen, Michael R. Lappin

Canine parvovirus typically infects puppies and young dogs with incomplete immunity, replicating in the nucleus of rapidly dividing cells of the lymphoid tissue, intestinal epithelium, and bone marrow with continued systemic circulation during infection (viremia). The provision of antibodies to those infected with a parvoviral viremia (passive immunotherapy) can inactivate some of those viruses undergoing replication, potentially lessening illness severity and decreasing mortality. Due to the significant gastrointestinal losses of albumin and protein resulting in hypovolemia, oncotic support is a commonly sought-after treatment for both stabilization and ongoing critical care management. Plasma (fresh or frozen) is a commonly used oncotic blood product, obtained through well vaccinated and screened community blood bank dogs who may have high levels of anti-parvovirus antibodies. The objective of this study was to measure anti-parvovirus antibodies from a convenience sampling of sera that had been previously saved at -80°C after testing Colorado State University blood bank dogs for flea and tick-borne pathogen exposure. Sera was selected sequentially from May 2021 to November 2023 based exclusively on whether an adequate volume for testing was available. The samples (n=49) were sent to the New York State Diagnostic Laboratory for determination of anti-parvovirus Type 2 antibody titers by hemagglutination inhibition. At the time of abstract submission, results from 48 samples had been completed with 39 dogs having high (1:640 – 1:5120), and 8 dogs classified as very high (≥1:10,240) titers, respectively. Studies to determine how the total amount of anti-parvovirus antibody in a unit of plasma with very high titers compared to the amount of anti-parvovirus antibodies delivered in other commercially available products are still ongoing. This study was supported by the Center for Companion Animal Studies.

Veterinary Tech/CS

96. Optimizing an enzyme-linked immunosorbent assay for transgelin-2 to diagnose feline infectious peritonitis

Lily Tees, Alora LaVoy, Gregg Dean

Feline infectious peritonitis (FIP) is a systemic disease that is devastating in young and immunocompromised cats. FIP is caused by feline infectious peritonitis virus (FIPV), which is thought to arise from a genetic mutation of the common feline coronavirus (FCoV) among other host and environmental factors. Diagnosis of FIP is challenging because it is usually accomplished post-mortem using immunohistochemistry (IHC) on formalin-fixed tissues. There is a need to develop a noninvasive ante-mortem diagnostic test to detect the disease and begin treatment before clinical signs worsen. Three plasma proteins have been identified through proteomic analysis as potential diagnostic biomarkers for FIP: neural cell adhesion molecule-1 (NCAM1), transgelin-2 (TAGLN2), and tropomyosin-4 (TPM4). The expression of these proteins was altered in cats with FIP compared to healthy cats and cats with other viral diseases. We hypothesized that enzyme-linked immunosorbent assays (ELISA) can be developed against the feline proteins and used in combination to identify cats with FIP. To achieve this, a TAGLN2 sandwich ELISA was optimized using recombinant feline TAGLN2 and plasma from healthy cats. Different concentrations and reagents for coating, detection, and secondary antibodies against TAGLN2 were tested in addition to multiple horse radish peroxidase (HRP) substrates until the strongest signal: noise ratio was achieved. An ELISA for feline NCAM1 has already been optimized and a TPM4 ELISA is under development. Future experiments will involve repeated screening of the original samples used in the proteomic study with the optimized assay for each protein, followed by a clinical trial to test plasma samples from cats with and without FIP. Research supported by Morris Animal Foundation. Student support provided by NIH training grant T35OD015130.

DVM Student/MIP

97. Cellular decapping enzyme Dcp2 implicated in the production of subgenomic flavivirus RNA

James S. Terry, Dionisius Denis, Brian Geiss

Flaviviruses, including West Nile and Zika viruses, are positive-sense single-stranded RNA viruses that pose a significant threat to global health. During the flavivirus infection cycle, capped viral RNAs are produced to allow translation of viral proteins and genome packaging into new virus particles. Capped viral RNA can be decapped by cellular decapping enzymes from the RNA degradation pathway to create monophosphorylated genomes that can be degraded by the Xrn1 exonuclease to produce subgenomic flavivirus RNA (sfRNA). sfRNA has been implicated in active Xrn1 depletion and further host cell immune downregulation, thus altering the intracellular environment to benefit virus replication and enhancing disease pathogenesis. Recent results from the Geiss lab suggest that the cellular Dcp2 decapping enzyme may be involved in the generation of sfRNAs. To further investigate Dcp2's role in this process, we reduced Dcp2 expression in human Huh7 cells using siRNA, infected cells with West Nile virus and purified RNA from both cells and virus particles. We used splinted ligation to specifically attach an adapter RNA only to the 5′ end of monophosphorylated viral genomes and then used quantitative reverse transcription polymerase chain reaction (qRT-PCR) to quantify the ratio of monophosphorylated and total viral RNAs present in the samples to establish if Dcp2 can generate monophosphorylated viral RNAs. We found that the proportion of monophosphorylated viral RNA in both cells and newly made virions decreased significantly in the absence of Dcp2, indicating that cellular Dcp2 is necessary for the production of monophosphorylated viral RNA in infected cells and virus particles. These results are impactful because they implicate a cellular RNA decay enzyme in the production of a viral RNA species known to be critical for viral pathogenesis. Student support provided by NIH T32AI162691.

Graduate Student/ MIP

98. Equine endometrial organoids as an in vitro model for endometritis to trial novel therapeutics

Riley E. Thompson, Mindy A. Meyers, Ahmed Gad, Gerrit J. Bouma, Christianne Magee, Budhan S. Pukazhenthi, and Fiona K. Hollinshead

A healthy uterine environment is essential for equine fertility. Approximately 20% of Thoroughbred mares demonstrate persistent breeding-induced endometritis (PBIE), creating a uterine environment that is unfavorable for pregnancy. Despite extensive *in vivo* studies, the exact mechanisms and best strategies for resolving PBIE remain elusive. A potential therapeutic for PBIE is extracellular vesicles (EVs), which are lipid-bound nanoparticles containing proteins and nucleic acids, secreted by equine embryo-derived mesenchymal stem cells (EDMSCs). We hypothesized that EVs secreted by EDMSCs contain anti-inflammatory miRNAs that prevent PBIE in susceptible mares. Using organoids as physiologically-relevant 3D cell cultures, our objectives were to assess i) the presence of specific anti-inflammatory miRNAs in EDMSC-derived EVs and ii) miRNA and mRNA of endometrial organoids (n=6 mares; Kenney-Doig score IIb) pre-treated with or without EVs 24 h prior to inflammatory stimulation with frozen-killed sperm (FKS), lipopolysaccharide (LPS), or PBS (Control) for 3 and 12h. Results demonstrated the presence of specific anti-inflammatory miRNAs (miR-10a, miR-21, miR-24, miR-145, and miR-146a) in EVs secreted by EDMSC cell lines. However, these anti-inflammatory miRNAs did not increase in the organoids after EV treatment ($p>0.05$). Treatment of organoids with FKS did not result in inflammatory gene expression differences compared to Control ($p>0.05$). Organoids pre-treated with EVs then stimulated with LPS demonstrated increased pro-inflammatory *IL1β* at 3h after LPS exposure and increased *PTGS2* expression at 12h after LPS exposure compared to Control organoids not pre-treated with EVs ($p<0.05$). Furthermore, organoids stimulated with LPS had increased expression of anti-inflammatory *IL1RN* (12h) and pro-inflammatory *IL6* (3 and 12h) compared to Control organoids not pre-treated with EVs. These data indicate that i) EDMSC EVs contain anti-inflammatory miRNAs, ii) anti-inflammatory miRNAs were not different in organoids treated with or without EVs, and iii) LPS was effective in stimulating inflammation in endometrial organoids, indicating suitability as an equine endometritis model. Research supported by the Grayson-Jockey Club Research Foundation.

Post-doctoral Fellow/CS

99. Characterizing abnormal cases of chronic wasting disease observed in Norwegian moose possessing a lysine to glutamine polymorphism in the prion protein at codon 109

M.L. Tyer, Julianna Sun, Sehun Kim, Alyssa Block, Sylvie Benestad, Glenn Telling

The first case of chronic wasting disease (CWD) in Europe was identified in Norway in 2016. In collaboration with the Norwegian Veterinary Institute, our group has identified the etiology of European CWD as independent from North American CWD. This represents an exciting opportunity to study naturally emerging strains of prion disease. Unique among Norwegian CWD cases are those identified in moose possessing a lysine-to-glutamine polymorphism at codon 109 (K109Q). Unlike cases of wild type moose CWD in Norway, CWD in K109Q moose displays unusual biochemical characteristics. Notably, isolates from these K109Q moose poorly infect our existing gene-targeted mouse model which has the endogenous mouse PrP replaced with cervid PrP (GtQ mice). Since isolates from wild type Norwegian moose CWD can infect our GtQ mice, this polymorphism is likely affecting the transmissibility of this abnormal strain of CWD. To model the pathogenesis of K109Q-CWD, we have designed a new knock-in mouse model that replaces the endogenous mouse PrP sequence with the moose PrP sequence containing the 109Q codon. Supplementing this investigation as we establish our K109Q mouse colony, we are interrogating the susceptibility of the K109Q polymorphism in a cell-based infection assay. The results of these studies will help shed light on the pathogenesis and biochemical characteristics of emergent CWD strains. Research support provided by NIH awards R01NS121682, R01NS109376, PO1-0011877A and P01AI077774. Student support provided by NIH training grant T32GM144856.

Graduate Student/ MIP

100. Investigating the Influence of Intra-articular Estrogen on Post-Traumatic Osteoarthritis Following Mechanical Anterior Cruciate Ligament Injury in a Mouse Model

Emily M. Van Zeeland, Travis Montoya, Erin Padayachee, Kelly S. Santangelo, Jeremiah T. Easley, and Katie J. Sikes

Following anterior cruciate ligament (ACL) injury, up to 87% of individuals develop post-traumatic osteoarthritis (PTOA) in their knee joint. While females are more prone to ACL injuries, the role of sex differences in PTOA development remains unclear. Murine models consistently show that males experience more severe and faster PTOA progression than females, demonstrated by exacerbated joint pathology, heightened pain-related behaviors, and reduced injured limb loading. Preliminary studies reveal differential estrogen-related signaling in the ACL proteome of female and male mice following ligament injury, prompting our hypothesis that estrogen plays a protective role against PTOA development. To delineate the specific role of estrogen within the stifle, we administered unilateral hindlimb intra-articular (IA) injections of estradiol-17β immediately after mechanical compression ACL rupture in male and female mice. Control mice received IA saline sham injections. Longitudinal mobility, behavioral, and weight-bearing assessments were conducted prior to ACL rupture and weekly out to five weeks post-injury. Data at each timepoint was normalized to pre-injury measurements. Statistical analyses employed three-way ANOVAs for group, sex, and time ($p<0.05$). Three weeks post-ACL injury, estrogen-treated female mice exhibited increased speed, mobility duration, and distance traveled compared to saline-injected mice. At four weeks post-injury, mice that received estrogen displayed superior weight-bearing on the injured limb compared to those that received saline, irrespective of sex. Overall, mice that received estradiol showed improved mobility parameters compared to mice that received saline. Ongoing investigations aim to characterize histologic and radiographic changes to fully characterize PTOA development. This study highlights the potential of estrogen modulation as a novel therapeutic approach to mitigate the progression of PTOA following ACL injury, offering new avenues for innovative interventions in the management of this debilitating condition. Student support provided by NIH MSTP training grant T32GM136628.

DVM/PhD Student/CS

101. Veterinary anesthesia: an opportunity to reduce the environmental footprint of clinical care

Lindsey Viola, Valeria Sanchez, Joedy Quintana, Danielle Scott, Gregg Griffenhagen, Colleen Duncan

Volatile anesthetics are potent greenhouse gases, therefore reducing their use is a well-recognized way for health systems to address their environmental footprint. While significant improvements have been made within human healthcare, there is relatively less movement in the animal healthcare sector. The purpose of this study was to gauge knowledge of and interest in the environmental impacts of anesthesia among veterinary professionals. A voluntary online survey consisting of 21 questions was distributed to veterinary professionals involved in the use of anesthesia via the American College of Veterinary Anesthesia and Analgesia listserv and through special interest groups on social media. Descriptive and comparative statistics were performed using Statistical Package for the Social Sciences. There were 150 respondents; 71% identified as veterinarians, 28% veterinary technicians, and 1% did not specify. In general, this survey highlighted an overall knowledge gap concerning the topic, with only 32% of respondents able to correctly identify inhaled anesthetic agents that contribute significantly to greenhouse gas emissions. There was, however, strong interest in taking action, as 97% of respondents agreed that there is opportunity for improvement in reducing the emissions associated with general anesthesia in veterinary medicine. Less than half of respondents had participated in continuing education regarding this topic, but 72% were interested in attending relevant continuing education. This study also highlighted opportunities for sustainability education beyond anesthesia, as the majority of respondents worked at a practice that did not have a sustainability initiative or were unaware of the existence of one. Overall, the results of this study illustrate both an interest amongst professionals in gaining more knowledge about sustainability in anesthesia and room for improvement in education for anesthesia professionals regarding their environmental impact. These findings are an important foundational step in guiding efforts to develop and disseminate educational resources on sustainable veterinary medicine.

DVM Student/ MIP

102. Investigating glial fibrillary acidic protein to diagnose and predict outcome in canine traumatic brain injury

Katy Walrond, Claire Tucker, Kelly Hall, Britta Siegenthaler, Ayla Mollen, Tracy Webb

Biomarkers can be leveraged as point-of-care tests that aid in accurately diagnosing and predicting outcomes after head injury and associated traumatic brain injury (TBI). In humans, the time course and prognostic value of the biomarker glial fibrillary acidic protein (GFAP) has been relatively well-established, with a blood test approved by the U.S. Food and Drug Administration in 2018. GFAP is expressed by astrocytes, and its expression is upregulated after injury to the central nervous system. In dogs, head injury and associated TBI are usually treated with supportive care due to a dearth of evidence-based research and challenges with definitively diagnosing TBI without advanced imaging. Although GFAP has been measured in dogs, it has not been assessed in dogs with head injury. This study is evaluating the utility of measuring serum GFAP levels in dogs after head injury to diagnose TBI and predict patient outcome (survival to discharge) non-invasively. Serum GFAP concentration will be measured from time of presentation to Colorado State University's Veterinary Teaching Hospital through 72-hours post-head injury. The study will also assess trends in GFAP levels with changes in patient neurologic status using validated veterinary scoring systems. Analysis will include ANOVA testing and identification of cut-points for diagnosis of TBI as compared to clinical gold standard and survival to discharge. If found to have satisfactory sensitivity and specificity, use of GFAP in canine trauma patients can support more informed patient care and enhanced translational applications. Research supported by the Koster Endowment. Student support provided by Boehringer Ingelheim Veterinary Scholar Program.

DVM Student/CS

103. Enhancing the antioxidant capacity of bovine embryos against oxidative stress using quercetin supplementation

Jessica Wittenstein, Ahmed Gad, Nico Menjivar, Dawit Tesfaye

Assisted reproductive technology (ART) has helped infertility issues in both human fertility clinics and agricultural settings. However, despite in vitro produced embryos averaging an 80% fertilization rate and a 75% cleavage rate, blastocyst rates rarely exceed 35%. This trend is due to both exogenous and endogenous stressors during embryo culture. Oxidative stress, which is caused by an imbalance between reactive oxygen species (ROS) and antioxidants within the embryo, remains a serious problem in current ART. In order to combat this stressor, the embryo utilizes the nuclear factor erythroid 2-related factor 2- kelch-like ECH-associated protein 1 (NRF2-KEAP1) pathway to prevent and repair damage caused by ROS. Here we aimed to investigate the impact of the flavonoid quercetin supplementation in protecting embryos from oxidative stress. This supplementation is expected to stabilize the NRF2 protein activity by inhibiting its ubiquitination by Keap-1 (NRF2 inhibitor). NRF2 will then be expressed and alleviate damage caused by intracellular ROS accumulation. To analyze quercetin's impact on mitigating oxidative stress, embryos were cultured with quercetin (10µm) in both an oxidative stressful environment (20% oxygen) and in a normal culture environment (5% oxygen). Blastocyst rates were then calculated from the number of oocytes put into culture media and two-way ANOVA was used for statistical analysis. Results show a trend of higher blastocyst rates in embryos supplemented with quercetin (29.5% 4.57) compared to DMSO (20.2% 3.22) and nontreated control groups (18.4% 1.54) ($p = 0.26$). Further analysis on the resulting blastocysts include expression of stress associated genes, ROS accumulation, mitochondrial membrane potential and total cell count. Overall, the current study will provide knowledge on the potential of quercetin supplementation in enhancing the antioxidant capacity of embryos to mitigate oxidative damage.

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Graduate Student/ BMS

104. Influence of pH and temperature on the adsorption of radiocesium on Prussian Blue coated detonation nanodiamonds

Megan Zaiger, Ralf Sudowe

The accident at the Fukushima Nuclear Power Plant in 2011 resulted in the generation of radioactively contaminated water which had to be stored on site for long periods of time. To deal with this waste, the reactor operator TEPCO has begun to release the water into the ocean after most of the radioactivity has been removed by the Advanced Liquid Processing System (ALPS) process. To ensure that all radioisotopes of concern have been removed from the water, it is necessary to quantify ultra-low levels of radiocesium. The currently available techniques for this measurement are very labor and time intensive. The need for efficient, rapid, and reliable methods for the determination of radiocesium in ocean water represents a critical gap that this work seeks to address. A new technique using Prussian blue coated detonation nanodiamonds to adsorb radioactive cesium from water samples is currently being studied in the Sudowe Lab. Adsorption to coated nanodiamonds was found to be very effective for drinking water samples. The current study focuses on the effect of varying pH and temperature on the adsorption behavior. The coated nanodiamonds were contacted with Cs-137 at pH ranging from 4-9 and at temperatures from 4°C to 50°C. A High Purity Germanium (HPGe) detector was used to measure the amount of Cs-137 remaining in solution after contact with the DND. The data was then used to calculate the percent adsorption based on the initial amount of Cs-137 present. The compared data was used to calculate adsorption rates and put into a scatter plot for analysis. The lowest adsorption, 95%, was found at a pH of 6.5, while the largest amount of radiocesium was adsorbed at a pH of 8.0 with 96.9%. Overall, differences in pH showed very little effect on the adsorption rate and the temperature study showed similar results. Research supported by the MAP ERC.

Graduate Student/ERHS

105. Ionizing radiation and mortality: unraveling neurocognitive risks in a worker cohort

Tony Zbysinski, John Rosecrance, Andreas Neophytou

Current radiation safety regulations are based on data and results from populations exposed to acute high dose radiation, with lack of strong evidence for low dose IR health effects. This study investigates ionizing radiation's (IR) impact on neurocognitive-related mortality risk among occupationally exposed workers, aiming to help bridge the gap with respect to chronic low dose IR research. The study will leverage data from the Fernald cohort of the U.S. Million Person Study (MPS), which has a population of workers who processed uranium and were exposed to chronic low dose IR and other chemical hazards. We will explore potential amplification of risk by chemical co-exposures and analyze mortality by job category. In this ongoing research, completed steps include data preparation and the development of a detailed research plan. The following co-exposures will be assessed for effect modification: NO2/HNO3, machining fluids, vehicle exhaust, welding fumes, TCE, asbestos dust, silica dust, coal dust, uranium dust, and tributyl phosphate and kerosene. Cox Proportional Hazards regression will be used to examine mortality risk for neurocognitive outcomes. Preliminary results may be available at the presentation. Results of this research will further our understanding of low dose IR and its effects on neurocognitive health outcomes, guide radiation safety policy, and inform work design decisions for occupational contexts where radiation and chemical exposures are both present. This work is supported by the Mountain and Plains Education and Research Center, a NIOSH education and research center for occupational and environmental health and safety.

Graduate Student/ ERHS

106. Assessment of wildfire smoke exposure on bovine reproductive health

Raquel Zisumbo, Sheryl Magzamen, Luke Montrose

Wildfires continue to increase in size and intensity, particularly in the Western United States (US). Individuals located close to wildfire sites are most at risk, but recent studies have demonstrated that the impact of wildfires can expand well beyond the wildfire site into surrounding states and countries. The Rocky Mountain region, including Colorado, is heavily impacted by local and distant wildfires. One constituent of wildfire smoke is fine particulate matter (PM2.5). PM2.5 is often used to measure smoke exposure effects due to its ability to reach the deep lung and cross the blood-gas-barrier. PM2.5 causes adverse health effects in the cardiopulmonary systems, and recent studies have indicated that it may negatively impact other systems within the body, such as the reproductive system. Cattle have been previously used as models for human reproductive health to further develop reproductive technologies such as artificial insemination, IVF, and embryo transfer. Further, cattle are plausible human sentinels given reproductive cycles, gestational lengths and life spans are relatively similar. Our long term goal is to determine the relationship between changes in PM2.5 and sperm quality metrics using data from bulls located in Bennett, CO from February 2021 through October 2023. Here, we will summarize descriptive statistics for this dataset including number of observations, sperm quality parameters, and local daily air quality values.

Graduate Student/ERHS

107. Epizootic hemorrhagic disease virus (EHDV) prevalence in white-tailed and mule deer in northeastern Colorado

Sophie J. Zook, Barbara A. Wolfe, George Wittemyer, Jeremy Alder, and Christie E. Mayo

Epizootic Hemorrhagic Disease Virus (EHDV) is a segmented *Orbivirus* transmitted by the *Culicoides* genus of midges. Wild ruminants are particularly susceptible to this disease, with white-tailed deer (*Odocoileus virginianus*) seeming to be more acutely affected than mule deer (*Odocoileus hemionus*). Infected white-tailed deer demonstrate hemorrhage and acute mortality, while male mule deer develop peruke (deformed) antlers with evidence of the virus causing sclerosis of testicular tissues. As white-tailed deer populations expand into Colorado from the Northeast, we expect an increase in both white-tailed EHDV-related deaths and male mule deer with peruke antlers in areas where EHDV is circulating. In this study, a total of 40 white-tailed and mule deer were captured and sampled from Weld and Larimer counties in Colorado. Serum samples were taken to test for the prevalence of EHDV from both male and females, and testicular aspirates were taken from males. These results will be important to identify the prevalence and epidemiology of EHDV in Colorado as the deer populations continue to change in our state. Research support was provided by USDA-NIFA-AHDR-COLV-23 and USDA-NIFA AFRI grant number 2019-67015-28982 as part of the joint USDA-NSF-NIH-BBSRCBSF Ecology and Evolution of Infectious Diseases program. Student support was provided by the National Institute of Food & Agriculture at the United States Department of Agriculture USDA-NIFA-AHDR-COLV-23.

DVM Student/ MIP

108. Clinical signs of HPAI in wild birds presented to US rehabilitation facilities

Giulia Ferrari and Barbara A. Wolfe

Highly Pathogenic Avian Influenza (HPAI) poses a significant threat to both domestic poultry and wild birds. In the past few years, an H5N1 strain of HPAI has emerged which is unusually adapted to wild birds, leading to high levels of mortality, particularly in wild raptors, corvids and waterfowl. In this study, we surveyed wildlife rehabilitation centers across the United States, representing all four flyways, for records of clinical signs in wild birds testing positive for H5N1. HPAI was diagnosed by PCR testing of tracheal or oropharyngeal swabs in commercial laboratories. Clinical reports from 14 wildlife rehabilitation centers represented 161 birds of 18 species, from the families *Anseriformes*, *Pelecaniformes*, *Accipitriformes*, *Strigiformes*, *Passeriformes*, *Charadriiformes* and *Falconiformes*. Clinical descriptions were categorized and compared across species to identify potential variations. Preliminary results indicate that the clinical signs of H5N1 in presented birds are consistent across the US and include primarily lethargy, anorexia, depression, corneal edema, nasal discharge, and neurological abnormalities. However, clinical signs of H5N1 appear to vary among avian taxa. This study was designed to assist wildlife rehabilitators, veterinarians, and researchers in understanding the clinical signs associated with HPAI in wild birds. Early detection and reporting can lead to prompt intervention, preventing the spread of the disease and mitigating impact on avian populations. A better understanding of the clinical signs of H5N1 will also aid identification of strain variants, distinction from other viruses, and eventually, variations of clinical signs in mammalian and avian species. Student support provided by USDA-NIFA-AHDR-COLV23.

DVM Student/CS

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